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GASTRIC ANALYSIS*

THE SECRETION CURVE AS DEVELOPED BY FRACTIONAL ANALYSIS

BY JOSEPH W. LARIMORE, A.B., M.D., ST. LOUIS, MO.

THE use of the stomach tube for diagnostic examination of the stomach contents received an impetus by the introduction and use of the gastroduodenal tube for securing the gastric contents before, and fractionally during the digestive cycle. The method has been extensively used during the past ten years and large contributions made to the study of the gastric function by Gros, Skaller, Palifaski, Bert, Fishbaugh, Hawk, and Rehfuss. The greater effectiveness of the small tube as compared to the large caliber tube in emptying the stomach and the facility of its clinical use are generally conceded. Recently, a number of writers have criticised the fractional method of gastric analysis. Wheelon,[1] Gorham,[2] Kopeloff[3] and White[4] have published articles to show that fractions of the stomach contents withdrawn periodically are not aliquot portions, and the method has on that premise been decried as not giving a dependable curve of the acidities during the digestive cycle. The practice of gastric analysis has fallen into greater disuse because of this. The improving and increasing use of the x-ray and its more direct visual approach in the examination of the stomach has also done much to discourage the practice of gastric analysis. The two methods should be used as supplementary.

Kopeloff claims that single determinations of gastric acidity by the Rehfuss method are not sufficient on which to base conclusions, because they do not take into consideration individual variation, and that the aliquot fractions are not representative. White concludes that only gross changes in acidity have clinical importance. He found that, without mixing, the av-

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erage variation of free HCl was 17 and of the total acid was 22, and that, with mixing, the average variation of free HCl was 10 and of the total acid was 8.5.

Wheelon suggests the following causes for the variations which he found in the samples: Shifting of the tube tip changes portion of the stomach from which the tube delivers; rapid emptying of the stomach alters degree of acid concentration; response of given stomach to a test meal may not be usual; sudden withdrawal may have an effect; and regurgitation of the duodenal contents may alter the gastric acidities. These factors can operate to disturb the validity of the samples as representative portions of the gastric contents; but, in the experience recorded in this paper they have not so operated with any disturbing frequency.

The procedure of the gastric analyses forming the material of this paper is as follows: The Rehfuss gastroduodenal tube is used. The patient is fasting and without fluid from the previous evening. The fasting gastric con-

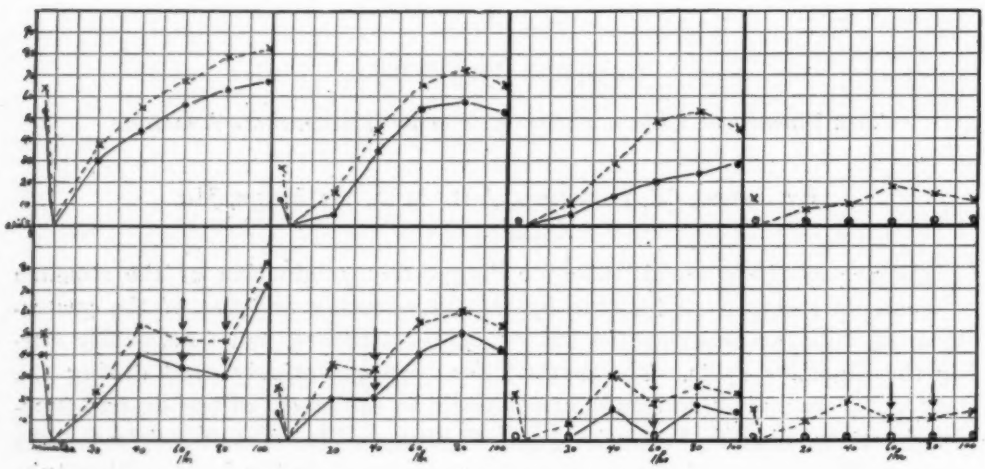

Fig. 1.—Selected Actual Curves.

Upper row shows curves with all samples valid.
Lower row shows curves with invalid titrations indicated by arrow. (See text.)

tents are aspirated, and the test meal is then taken. The test meal consists of one shredded wheat biscuit (ground) and 400 c.c. of water (two tumblers), with 1 c.c. of phenolsulphonephthalein. Aspirations of 10 c.c. samples are made every twenty minutes for five samples and with the fifth the stomach is emptied. Before and after each aspiration one syringe full of air is injected. The fasting contents are measured, examined, and titrated. Each sample is titrated and examined. The last sample is measured.

Two hundred and fifty-seven cases were subjected to 275 analyses. This experience shows that the curves of the gastric secretion as revealed by fractional analysis show an ascending gradient followed by a descending gradient and that accidental variations of the fractions aspirated are not of a degree or frequency as to vitiate these curves. Among these 2487 titrations of 1244 samples in 275 curves, there appears a contrary variation from this usual gradient, by one sample in 123 curves, and by two samples in 29 curves, and

in only 9.1 per cent of the titrations. Such conformation to the curve gradient would seem to contradict the results of White, Wheelon, and Gorham.

Fractional gastric analysis as practiced does not reveal the physiology of the stomach in all its separate phases and does not exhibit these in an absolute evaluation, but rather the curve of the fractional analysis is the composite resultant of the present (occasional) factors in the physiologic status of the stomach, as modified by the artificial standardized factors introduced (dilution, tube presence, nonpalatable meal, aspiration and air injection) into the test.

Samples are considered valid if their titration value follows the direction of the curve in its normal ascent and descent. A sample is considered invalid if the titration value fails to follow the curve direction, by being equal to the titration immediately preceding, or if it reverses the gradient of the curve. Persistence of the zero of achlorhyrdia is not considered as invalidating the samples for HCl. Then the titration of the total acid only shows discrepancies. Samples are invalidated for failure either in the curve of free HCl or in that of total acid. A high titration in the first sample is considered as a failure to aspirate entirely the fasting contents and is always associated with a higher free acid in the fasting contents. Table I summarizes the percentage incidence of invalid samples.

TABLE I
SUMMARY

	TOTAL	
Cases	257	
Curves	275	
Titrations of fractional samples	2487	
Titrations falling into invalid position on curves	227	9.1%
Curves having one or more invalid samples	123	44.8%
Curves having two invalid samples	29	10.5%

TABLE II
TYPE OF CURVES

	TOTAL	HYPERACIDITY	FASTING HYPERACIDITY NORMAL	NORMAL	HYPOACIDITY	ACHLORHYDRIA	MALE	FEMALE
Total	257	42	23	89	71	32	115	142
Duodenal Ulcer	45	28	4	10	2	1	39	6
Gastric Ulcer	6	1	–	2	2	1	–	6
Gastric Cancer	2	–	–	–	–	2	2	–
Gastric Syphilis	1	–	–	1	–	–	1	–
Gall Stones	12	–	1	5	5	1	2	10
Gall bladder Disease	25	–	1	9	12	3	4	21
Appendicitis, Chronic	23	1	1	9	7	5	9	14
Gastritis, Chronic	7	2	–	3	–	2	4	3
Gastric Motor Delay, functional	3	–	–	3	–	–	2	1
Dyspepsia Nervosa	28	2	4	11	10	1	17	11
Migraine	5	–	1	1	3	–	–	5
Enteritis, Acute	5	–	1	1	3	–	–	5
Constipation, Chronic	25	2	2	13	8	–	9	16
Colitis	11	1	1	5	2	2	4	7
Tuberculosis, Pulmonary	4	–	–	2	1	1	3	1
Anemia, Simple	1	–	–	–	–	1	–	1
Neurasthenia	11	1	2	5	1	2	4	7
Autonomic Imba'ance	7	–	–	1	2	4	3	4
Cirrhosis of the Liver	4	1	1	–	1	1	3	1
Other Diagnoses	32	3	4	8	12	5	9	23

In tabulating these curves to show the relation of the secretory activity to various conditions, they have been classified according to the highest hydrochloric acid concentration reached during the period. Hyperacidity indicates a value of HCl greater than 50 and does not mean the secretion of an acid of an abnormally high concentration. Normal acidity indicates values between 30 and 50. Hypoacidity indicates positive values under 30, and achlorhydria indicates the continued absence of free HCl.

Curves showing a hyperacidity in the fasting contents with a secretory curve within normal limits were made a separate group. Hyperacidity curves were of significant greater frequency in duodenal ulcer; gall bladder and appendix syndromes show a definite tendency to hypoacidities.

In the twelve cases having two or more gastric analyses there was a correspondence in the repeated curves, or a variation consistent with the clinical and x-ray findings in the cases. Two cases of duodenal ulcer showed, in two and three curves respectively, an increase in the acidities corresponding to the improvement of gastric motility as shown by x-ray. One case of acute gastroenteritis showed an increase in acidities of the second curve corresponding to the clinical and x-ray improvement. One case showing an achlorhydria initially showed a hypoacidity in an early repeated curve which was made because of a suggested psychic inhibition of secretion. This small experience in repeated curves has shown either a lack of variation or a logical correspondence to the clinical progress.

DISCUSSION

This analysis of a clinical experience with fractional gastric analysis gives an assurance in its validity for demonstrating the physiologic response of the stomach to the standardized factors of the method. The valuable data of gastric secretion have not been better developed than by this method. It does demonstrate alterations in gastric secretion and motility which are more or less directly diagnostic and helpful therapeutically. The demonstration of an achlorhydria is prompt and conclusive by this method. The extent to which the data developed by this method of gastric analysis may be diagnostically or therapeutically useful is more limited than has been claimed for practical deductions from analogous data in the past.

Gastric analysis cannot be discarded as being altogether superceded or replaced by newer methods of gastrointestinal examination. The examination of the fasting contents of the stomach is essential to gastrointestinal diagnosis, and the observation of the curve of the gastric acidities by the slow fractional method may give useful and necessary information. Knowledge of the gastric acidities has been of the greatest help in selecting a diet especially with consideration of the buffer values of various foods.

White asks if the practice of fractional gastric analysis is worth its time and effort. There is a confusing multiplicity of present clinical diagnostic procedures. Time alone, not to mention the wear and tear upon a patient, demands a thoughtful selection. Observation of the fasting gastric contents and the demonstration of active or absent hydrochloric acid secretion are essential. The demonstration of an achlorhydria has high clinical value in

other than gastrointestinal conditions. It occurred in 12.2 per cent of this series. Vanderhoof,[5] in a series of 4281 patients subjected to gastric analysis, shows an incidence of achylia gastrica of 10.5 per cent. He emphasizes that "there is good reason to believe that every person with true achylia gastrica has a potential case of either pernicious anemia or combined spinal sclerosis." The demonstration of hydrochloric acid in the fasting contents may obviate the need for developing the secretory curve where the x-ray is used for demonstrating gastric motility and organic changes.

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THE LEVINSON TEST AND OTHER LABORATORY STUDIES IN TUBERCULOUS MENINGITIS*

By C. A. Pons, M.D., and Thelma A. Fletcher, Philadelphia, Pa.

THE typical cerebrospinal fluid in tuberculous meningitis is generally clear or slightly turbid, increased in amount, and under increased pressure. The globulin is increased, there is a moderate increase in the leucocytes with the small lymphocyte greatly predominating, and the sugar content is within normal limits. These findings do not per se confirm the diagnosis of tuberculous meningitis, nor do they exclude other possibilities such as cerebrospinal lues, paresis, encephalitis, poliomyelitis, etc. Further than this, cases of tuberculous meningitis have been reported in which the predominating cell was the polymorphonuclear leucocyte; hence in these rare cases we meet the possibility of confusing the condition with a purulent meningitis, providing the bacteriologic examination is negative, as sometimes happens, especially in epidemic meningitis.

We have various tests that help in differentiating some of the conditions resembling tuberculous meningitis, the Wassermann reaction and the colloidal gold would almost rule out lues, a high sugar content will usually exclude encephalitis, etc. But these are time consuming and difficult except for the experienced worker and often not available.

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The crucial test in proving that the fluid in question is tuberculous is, of course, the finding of the acid-fast organism. This is not an easy task and frequently unsuccessful and although, as a last resort a guinea pig inoculation can be made, the report is necessaarily delayed five to six weeks.

Various procedures have been suggested for finding tubercle bacilli. Allowing the fluid to stand undisturbed overnight in an incubator is supposed by some to increase the number of organisms, and others advise the addition of an equal volume of 95 per cent alcohol when there is no pellicle. While the pellicle is often present in tuberculous spinal fluids, many have the notion that it is only found in tuberculosis. This impression we want to correct, as we have seen pellicles in other conditions. When, however, a pellicle does form, it enhances our chances of finding the bacillus.

Of the various chemical tests that have been suggested, we have found the precipitation test of Levinson[1] extremely valuable. Proteins are precipitated as albuminates when treated with certain metallic salts, such as copper sulphate, zinc chloride, bichloride of mercury and so forth, and precipitated as insoluble salts when treated with weak organic acids, such as certain of the alkaloidal reagents, picric, phosphotungstic, tannic, and sulphosalicylic acids.

Levinson claims that in cases of suppurative meningitis the sulphosalicylic acid throws down a precipitate, the height of which in millimeters is three times as great as that thrown down by the mercuric chloride, whereas in tuberculous meningitis the reverse is the case, i. e., the bichloride precipitate is twice that of the sulphosalicylic acid.

While the protein content of the fluid in cerebrospinal meningitis is usually greater than in tuberculous meningitis, the range according to Mestrezat[2] being from 0.15 to 0.85 per cent for epidemic meningitis and from 0.13 to 0.56 per cent for tuberculous, Tashiro and Levinson[3] concluded, after carefully conducted experiments, that the test did not depend on the relative amounts of protein present, but on the hydrogen-ion concentration.

The changes in the hydrogen-ion concentration of the fluids on standing was investigated and a difference was noted, which was in itself sufficient to alter the electrical charges of the proteins. They found in epidemic meningitis that most of the proteins are positively charged, while in tuberculous meningitis the negatively charged proteins predominate. Hence, the more positively charged proteins present in epidemic meningitis give a higher precipitate with the sulphosalicylic acid which is negatively charged, than with the bichloride of mercury which is positively charged, the reverse being the case in tuberculous spinal fluid. This phase of the problem was not investigated by us.

We have used various alkaloidal metallic precipitants, but the results at best equal those obtained with the two reagents proposed by Levinson. We have used various dilutions of the spinal fluid, and various strengths of the reagents. In very cloudy spinal fluids it might be advisable to use various dilutions of the spinal fluid. The merits of the stronger solutions, along with the original test as proposed by Levinson will be given at length as we describe our work.

A study of tuberculous meningitis was undertaken with this test in view. We found that in a number of cases in which we were able to establish the presence of the tubercle bacilli in the spinal fluid, either before death or at autopsy, the one to two ratio of the two precipitates held good in practically all of our cases, furthermore, that in most of the cases we got this one to two ratio before finding the bacillus.

The following routine was observed: The spinal fluid was received in two sterile test tubes, one being used for cell count, differential, and precipitation test. In doing this test at first, we followed Levinson's technic. Small test tubes of about 8 mm. in diameter were used. We placed one cubic centimeter of spinal fluid in each of two tubes. To one we added one cubic centimeter of a 1 per cent mercuric chloride and to the second one cubic centimeter of a 3 per cent sulphosalicylic acid. The tubes were shaken well, stoppered and stood at room temperature for twenty-four hours. At the end

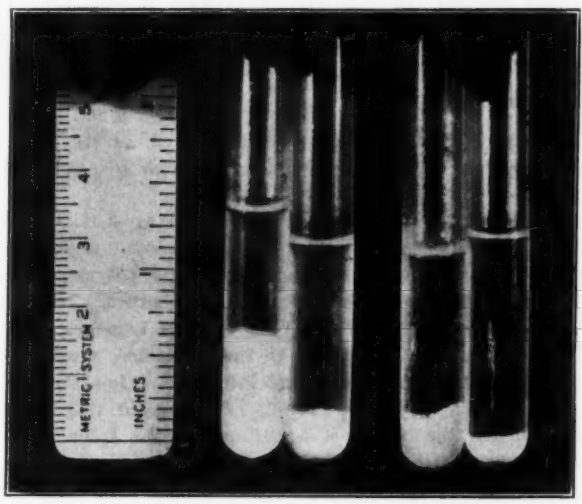

Fig. 1.—Right to left: 1. Mercuric chloride; 2, sulphosalicylic acid, three days before death. Ratio 1:3+. T. B. found; 3, mercuric chloride; 4, sulphosalicylic acid, one week before death. Ratio 1:2+. No T. B. found.

of that time the column of precipitate was measured in millimeters. The second tube of spinal fluid we left undisturbed, until a pellicle had formed. This usually took place in less than twelve to fourteen hours. The pellicle was then fished out, teased out well, and stained for tubercle bacilli in the routine manner. When no pellicle was formed, we centrifuged at high speed for one-half hour. This sometimes forms a small pellicle, or else by the addition of 95 per cent alcohol, we precipitated the proteins and on centrifuging a compact sediment results. Occasionally, we had to resort to a guinea pig inoculation.

The two precipitates are of an entirely different character, that of the sulphosalicylic acid is heavy and compact and starts to form immediately, while that of the mercuric chloride is light, feathery, and forms slowly. Sometimes the precipitate does not come down into a compact sediment. It seems as if little floccules become adherent to the walls of the test tube. We

found it generally advisable to shake the tubes two to three hours before making the final reading. If one bears in mind that it is not the amount of protein thrown down in the two precipitates but the relative height of the column of protein in millimeters in each tube, the results are more uniform.

Another difficulty encountered was that in some cases we did not obtain a precipitate, but the tube remained cloudy. It was thought that in these cases either lower dilutions of the fluid or stronger reagents would avoid this difficulty. Better results were obtained by the use of stronger reagents, and since then we use 1 per cent and 2 per cent bichloride and 3 per cent and 6 per cent sulphosalicylic acid solutions.

With reference to the use of these higher strengths an analogy with the use of the cholesterin antigen in the Wassermann reaction seems permissible. The use of the cholesterin antigen alone would no doubt increase the number of false positive reactions, as well as the strength of the positive test, yet no serologist at present would do without it, using a less sensitive antigen as well. The same applies to the use of the stronger solutions in this test. It is valuable in borderline cases and especially where the lower solutions fail to bring down a sediment.

The results of our investigations are given in Tables I, II, III, and IV, but only those atypical or interesting cases will be given in detail.

J. R., an adult male was admitted to the Psychopathic Wards of the Philadelphia General Hospital. The provisional diagnosis was lethargic encephalitis. Study of the cerebrospinal fluid showed 217 cells per cu. mm. of which 4 per cent were polymorphonuclears and 96 per cent lymphocytes. No organisms were found on smear or culture. The Wassermann and the colloidal gold excluded syphilis of the nervous system. The Levinson test gave a precipitate of 3 mm. in the sulphosalicylic acid and 9 mm. in the bichloride, a ratio of 1:3. Because of this, a guinea pig was injected and five weeks later the animal was autopsied. Typical lesions were found and tubercle bacilli recovered. The patient was removed to the Jewish Hospital of Philadelphia, and the organism was found in the spinal fluid. This case illustrates the diagnostic value of the test. It is unfortunate that we have not obtained any fluids from cases of lethargic encephalitis.

J. L., an adult male was admitted to the Men's Tuberculous Wards of the Philadelphia General Hospital with definite symptoms of pulmonary tuberculosis. Later the patient developed symptoms and signs of meningitis. On the first lumbar puncture fluid the Levinson test was indicative of tuberculous meningitis, but no organisms were found. Three days later, another puncture was done, the Levinson ratio was 1:3.4, and this time we were successful in finding the tubercle bacilli.

This case illustrates a not uncommon experience, namely, that the Levinson test is generally positive before the organism can be found.

R. W., an adult male. This patient was admitted to the Medical Wards of the Philadelphia General Hospital with tuberculous cervical adenitis and symptoms and signs suggestive of meningitis. This case is reported because of the interesting cytology.

COUNT	CELL COUNT	POLYS PER CENT	LYMPHS PER CENT	RATIO OLD SOL.	RATIO NEW SOL.	T. B.
1	1350	75	25	1:2.5	1:2.9	–
2	56*	29	69	1:1.75	1:2.5	+
3	430	25	75	1:2.5	1:2.8	–

*Pellicle present.

TABLE I

LEVINSON TEST AND SPINAL FLUID FINDINGS IN TUBERCULOUS MENINGITIS

CASE	HEIGHT IN MM.		RATIO	CELL COUNT	DIFFERENTIAL			CEREBROSPINAL FLUID		T. B. IN SPINAL FLUID	AUTOPSY FINDINGS (BRAIN)
	Sul. 3 Per Cent	Hg Cl_2 1 Per Cent			Polys. Per Cent	Endo. Per Cent	Lymphos. Per Cent	Wassermann	Colloidal Gold		
J.L.	5	17	1: 3.4	130	3	1	96	–	0011123210	–	0
				183	10	9	81			+	
T.H.	10	22	1: 2.2	156	–	–	100	–	2534444443	–	P.M. Military T.B.
C.H.	4	9	1: 2.3	75	–	–	100	–	None Made	–	
C.H.	5	13	1: 2.6	21	–	–	100	–		–	0
										P.M.L.P. +	
J.B.	8	34	1: 4.2	25	–	–	Predominate	–	None Made	+	0
J.S.	4	8	1: 2.0	371	15	–	85	–	1245555433	–	
J.S.	4	11	1: 2.8	–	–	–	–			–	Yes
										P.M.B.S.+	+
E.G.	2	33	1:16.5	255	46	8	46	–	3455555320	–	
E.G.	4	32	1: 8.0	64	2	10	88			–	Yes
										P.M.B.S.+	+
W.Y.	3	5	1: 1.7	235	1	11	88	–	2445555511	–	
W.Y.	4	9	1: 2.3	182	2	8	90			+	Yes +
J.C.	5	20	1: 4.0	170	24	7	69	–	0013233222	–	
J.C.	–	–	–	316	8	3	79			+	Yes +
W.O.	6	20	1: 3.3	160	1	–	99	–	0001233311	–	
W.O.	6	18	1: 3.0	182	8	2	90			–	Yes +
J.R.	3	9	1: 3.0	217	4	18	78	–	0012221000	–	0
										G.P.+	
A.C.	2	4	1: 2.0	42	8	9	83	–	1233422100	–	Yes +

– Negative, or none found.
0 Not made.
P.M. Postmortem.
P.M.L.P.—Postmortem Lumbar Puncture.
P.M.B.S.—Postmortem Brain Smear.
G.P.—Guinea Pig Inoculation.

TABLE II

OLD AND NEW LEVINSON TEST AND SPINAL FLUID FINDINGS IN TUBERCULOUS MENINGITIS

CASE	HEIGHT IN MM.		RATIO OLD	HEIGHT IN MM.		RATIO NEW	CELL COUNT	DIFFERENTIAL COUNT			CEREBROSPINAL FLUID		TUB. BAC. IN SPINAL FLUID	P.M. FINDINGS BRAIN
	Sul. 3 Per Cent	Hg Cl_2 1 Per Cent		Sul. 6 Per Cent	Hg Cl_2 2 Per Cent			Polys. Per Cent	Endo. Per Cent	Lympho. Per Cent	Wass.	Colloidal Gold		
H.M.	3	8	1:2.7	3	12	1:4	52	–	–	Majority	–	0000111100	–	Yes
	3	6	1:2	4	14	1:3.5	75	4	10	86			–	+
J.G.$_1$	5	11	1:2.2	6	21	1:3.5	153	2	10	88	–	None made	–	Yes
J.G.$_2$	5	10	1:2	7	16	1:2.3	180	4	4	92			–	+
R.H.	3.5	5	1:1.4	4	19	1:4.7	162	–	17	83	–	None made	+	0
W.O.	6	20	1:3	Insuff.	27	?	160	1	–	99	–	0001233311	–	Yes
J.C.$_1$	2	Cloudy	?	2	6	1:3	Not Made	–	–	–	–	0001110000	–	+ Yes
J.C.$_2$	3	9	1:3	5	20	1:4	177	3	12	85			–	+
W.N.	3	Cloudy	?	3	9	1:3	115	6	3	91	–	2334432110	+	Yes
C.O.	12	Cloudy	?	12	24	1:2	500	2	4	94	–	None made	–	+ 0
R.G.	6	Cloudy	?	6.5	20	1:3	133	5	8	87	–	1111232200	G.P.+ – P.M.L.P. + –	0
M.G.	4	Cloudy	?	5	16	1:3.2	40	10	–	90	–	0000133210	P.M.L.P. +	Yes +
W.R.$_1$	4	10	1:2.5	4.5	13	1:2.9	1350	75	–	25	–		–	
W.R.$_2$	4	7	1:1.75	4	10	1:2.5	56	29	2	69	–	3555555321	+	0
W.R.$_3$	3	7.5	1:2.5	4	11	1:2.8	430	25	–	75			–	

TABLE III

LEVINSON TEST AND SPINAL FLUID FINDINGS IN CEREBROSPINAL SYPHILIS

CASE	HEIGHT IN MM.		RATIO	CELL COUNT	CEREBROSPINAL FLUID	
	Sul. 3 Per Cent	$HgCl_2$ 1 Per Cent			Wass.	Colloidal Gold
J.J.	2	4	1:2	–	++++	5555554200
L.R.	3.5	6.5	1:1.8	107	++	5555543100
L.R.	4	5	1:1.3	89	++++	5555554310
T.N.	4	5	1:1.3	94	++++	5555555554
W.C.	3.5	5	1:1.3	7	++++	5555553100
R.B.	5	5	1:1.0	20	++++	5555555544
N.B.	3.75	4	1:1.1	7	++	5555554310
S.G.	3	4.5	1:1.5	94	++++	5555555511
E.N.	3	5	1:1.7	40	++++	5555555422
D.U.	3	6	1:2			
	3	4	1:1.3	393	++++	5555553311
T.S.	2	5	1:2.5	275	++++	1122110000
M.S.	4	5	1:1.3	1000	++++	None made –

TABLE IV

OLD AND NEW TEST AND SPINAL FLUID FINDINGS IN CEREBROSPINAL SYPHILIS

CASE	HEIGHT IN MM.		RATIO OLD	HEIGHT IN MM.		RATIO NEW	CELL COUNT	CEREBROSPINAL FLUID	
	Sul. 3 Per Cent	$HgCl_2$ 1 Per Cent		Sul. 6 Per Cent	$HgCl_2$ 2 Per Cent			Wassermann	Colloidal Gold
E.B.	5.5	6	1:1.0	5	7	1:1.4	100	++++	5555555554
H.H.	7.5	11	1:1.5	7	14	1:2.0	108	++++	5555555433
E.C.	2	4	1:2.0	2	5	1:2.5	22	++++	2444321000
L.O.	4	3.5	1:1.1	4	5	1:1.3	80	++++	None Made
E.S.	5	6	1:1.2	6	7	1:1.2	24	++++	5555555542
C.B.	3	4	1:1.3	4	6	1:1.5	286	++++	2223442000
F.W.	3.5	8	1:2.3	4	11	1:2.8	23	++++	2224321000
F.M.	4	9	1:2.5	4	14	1:3.5	30	++++	4555544211
J.S.	5	7	1:1.4	5	8	1:1.6	78	++++	5555555543
W.R.	3	3	1:1	3	5	1:1.7	118	++++	4444311110
J.F.	3	4	1:1.3	4	7	1:1.8	131	++++	4444431000
							1210		
H.C.	3	Cloudy	?	3	Cloudy	?	P 99	++++	2333442100
	4	Cloudy	?	2.5	2	1:3.1	L 1		

In this case the first spinal fluid showed 75 per cent polymorphonuclears and at this time the case might have been considered a purulent meningitis, yet the ratio, sulphosalicylic acid to bichloride, was 1 : 2.9, suggesting tuberculous meningitis. In the next count the polys were diminished to 25 per cent. We had another case, E. G., Table I, in which there were 46 per cent polymorphonuclears and 46 per cent lymphocytes. This predominance of polymorphonuclears sometimes happens in tuberculous meningitis.

DISCUSSION

Results obtained with the Levinson test were gratifying with one exception, in the twenty cases of tuberculous meningitis reported. The ratio was 1 : 2 or above.

Furthermore, by using this test, we were encouraged to double our efforts in searching for tubercle bacilli and since success in finding the organism is almost in direct proportion to the efforts of the examiner, the test, in this sense, is also valuable.

TABLE V

LEVINSON TEST IN MISCELLANEOUS CONDITIONS

DIAGNOSIS	HEIGHT IN MM.		RATIO	HEIGHT IN MM.		RATIO	CELL COUNT	DIFFERENTIAL			CEREBROSPINAL FLUID	
	Sul. 3 Per Cent	$HgCl_2$ 1 Per Cent		Sul. 6 Per Cent	$HgCl_2$ 2 Per Cent			Poly. Per Cent	Endo. Per Cent	Lympho. Per Cent	Wassermann	Colloidal Gold
J. B. Pneumococcus meningitis after serum given	24	19	1.26:1	–	–	–	75	95	–	5	0	0
W. A. Pneumococcus meningitis after serum given	30	19	1.57:1	–	–	–	8,300	99	1	–	0	0
M. T. Pneumococcus meningitis before serum given	15	11	1.36:1	–	–	–	3,800	93	5	2	0	0
J. H. Meningococcus meningitis after serum given	5	3	1.66:1	–	–	–	6,700	78	22	–	0	0
M. R. Staphylococcus meningitis before serum given	3	Cloudy	?	4	5	1:1.2	830	100	–	–	Neg.	0001122100
F. P. Staphylococcus meningitis. Serum given between 2 tests	3.5 5	Cloudy Cloudy	? ?	4 7	7 7	1:1.7 1:1	1,240 1,660	99 99	– –	1 1	0	0
F. B. Influenza meningitis before serum given	5	Cloudy	?	4	13	1:3.5	5,800	100	–	–	Neg.	1111122000
E. McL. Influenza meningitis. Serum given	4 5 7 5	Cloudy 11 Cloudy 7	? 1:2.2 ? 1:1.4	– – – –	– – – –	– – – –	3,180 30 340 10,000	100 – 73 95	– – 10 –	– – 17 5	0	0
J. C. Encephalitis	4	7	1:1.7	–	–	–	12	–	–	–	Neg.	000C000000
W. McQ. 1. Glioma. 2. Basal meningitis	4.5	5	1:1.1	–	–	–	1,200	47	–	53	Neg.	000C000000

RESUMÉ OF TABLES I, II, III AND IV

		LESS THAN 1:2 RATES	1:2	1:2 TO 1:2.5	ABOVE 1:2.5	TOTAL
OLD TEST	T. B.	1	1	4	11	17
	C. S. LUES	17	3	3	0	23
NEW TEST	T. B.	0	1	–	9	10
	C. S. LUES	8	1	–	3	12

The 1 : 2 ratio was often established before the finding of the organism, while in other cases, in which the organism was not found, we had to either inject a guinea pig, or await postmortem findings for confirmation.

In three cases in which the tentative clinical diagnosis was not tuberculous meningitis, because of the results obtained with the precipitation test, we either made smears or injected a guinea pig, and thus independent of knowledge of signs and symptoms, or of a request for a bacteriologic examination, we made a diagnosis of tuberculous meningitis.

Before we began to use the stronger solutions, several cases came to observation in which, because no precipitate was obtained in the 1 per cent bichloride, further investigations to prove the cases tuberculous meningitis were discontinued. These cases were, of course, not included in this study. We believe that, with the stronger solution, this difficulty will be avoided and results will be more uniform.

Unfortunately, the 1:2 ratio is not pathognomonic of tuberculous meningitis, as 6 cases of cerebrospinal syphilis out of 24 studied (25 per cent) gave a ratio of 1 : 2 or over. If, however, we consider a ratio above 1 : 2 as proper for tuberculous meningitis, which, in the light of our work, seems permissible, the value of the precipitation test in differentiating between cerebrospinal syphilis and tuberculous meningitis is increased. Then, on the basis of our rather short series, one out of 10 cases would give a 1 : 2 ratio (10 per cent); but, obviously, in a study of 10 cases of cerebrospinal syphilis and 10 of tuberculous meningitis, we face only an error of 5 per cent.

No definite conclusions can be drawn as to the behavior of the test in the other conditions given in Table V, as the number of cases were not sufficient to warrant any. Practically always a higher precipitate was obtained in the sulphosalicylic acid than in the bichloride solution.

SUMMARY

We have confirmed Levinson's findings in that with his test the height of the precipitate in the bichloride of mercury solution is twice that of the sulphosalicylic precipitate.

A 1 : 2 ratio is very suggestive, but not pathognomonic of tuberculous meningitis.

The use of stronger reagents along with those proposed by Levinson is suggested as increasing the diagnostic value of the test.

The test should be performed in every case of meningitis, because (a) it is easy to do, (b) it is inexpensive, and (c) it gives valuable diagnostic information.

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STUDIES ON THE ACTION OF HISTAMINE ON HUMAN GASTRIC SECRETION*

By Louis M. Gompertz, M.D., New Haven, Conn., and
Martin G. Vorhaus, M.D., New York, N. Y.

HISTAMINE has been used for a long time in pharmacologic laboratories. It has been found in the human intestines by many observers, some of whom attach little or no importance to its presence, while others believe that a large variety of disorders are associated with this compound.

When histamine is injected intravenously into animals, a marked drop in blood pressure is the most prominent effect noted. In animals with a diminished adrenal supply, the lowering of the blood pressure is much more marked and death may ensue as a result of the action of histamine. The pressor action of epinephrin, pituitrin and similar drugs is inhibited by an intravenous injection of histamine.

When injected subcutaneously into animals, this substance produces a stimulation of gastric secretion, sweating, salivation, and congestion of the mucous membranes with little or no drop in blood pressure.

Histamine (Iminazolylethylamin) is an amin derived from the amino-acid histidin, a cleavage product of the proteins. The production of this substance is believed to be due to a decarboxylization of the amino-acid brought about by bacterial action. This theory is supported by the work of Mellanby and Twort and by Berthelot and Bertrand, who isolated organisms capable of converting histidin into histamine.

Ivy and McIlvain applied histamine locally to the intestines of dogs in order to determine the possible reflex action on gastric secretion. When a 1:1000 solution of histamine was applied to the duodenal mucosa for twenty to thirty minutes, a marked secretion of gastric juice occurred regularly and without fail. There was a latent period from ten to thirty minutes.

Because of the observed effect of histamine on the gastric secretion Carnot, Koskowski, and Libert conducted a study of the gastric secretory response to histamine on human subjects. Subcutaneous injections of hista-

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mine were given to fourteen individuals, and an increased amount of gastric secretion was observed.

Matheson and Ammon reproduced this work on a small group of normal subjects using ergamine acid phosphate which contains approximately one-third of the histamine base. This work was very carefully carried out and errors by psychical stimulation of secretion were eliminated as far as possible. The results practically substantiated the previous conclusions showing an increased flow of gastric juice without any untoward effects in man.

Early in the spring of 1923 we planned our experimental work with histamine. On the basis of earlier reports on normal subjects, we proceeded to repeat the previous investigations and to study the gastric response to

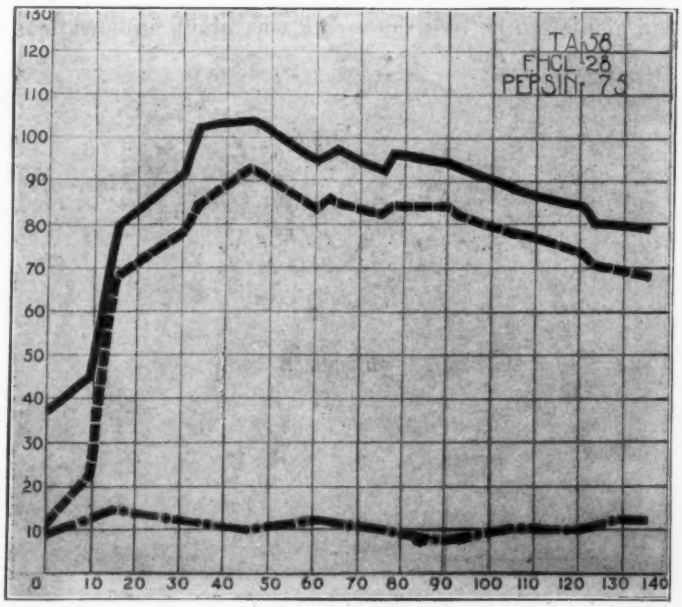

Fig. 1.—Showing a normal acidity after the Ewald test and a prolonged and increased response after the histamine test.
——— Total acidity; – – – – – – – – free hydrochloric acid; –.–.–.–.–.–.– pepsin (mm. digested).

histamine in various disorders of the stomach. We were interested in cases of achylia gastrica, in particular, because from a theoretical standpoint the reaction to this compound promised to offer a means of differentiating between true achylia, i.e., achylia with destruction of the secretory glands, and pseudo-achylia in which usually a functional inhibition of secretion occurs. In order to take into account the possible production of gastric secretion through irritation which might arise from the long continued presence of a tube within the stomach, the tube was allowed to remain in situ for one hour, the contents being removed at regular intervals before the injection of histamine. In no instance was any appreciable increase of secretion observed in this following manipulation.

In a number of patients saline solution was injected subcutaneously as

a control procedure and the stomach contents examined before administration of histamine. In all cases the effect of the saline injection was negative.

After satisfying ourselves that no increase of gastric secretion occurred by our method of investigation either from the stomach tube or injection per se, we proceeded as follows:

Forty-eight hours before the test an Ewald-Boas test meal was given to permit observations on the gastric phenomena, when no drug whatever was taken. No food or drink was given to the patients between supper the night before and the morning of the test. Early in the morning an Einhorn or Rehfuss tube was passed and the stomach contents were extracted with a Record syringe. In order to insure complete emptying of the organ the patient assumed different positions. After an interval of fifteen minutes the withdrawal of the fasting contents was repeated whereupon one c.c. of a 1:1000 solution of histamine hydrochloride was given subcutaneously. There-

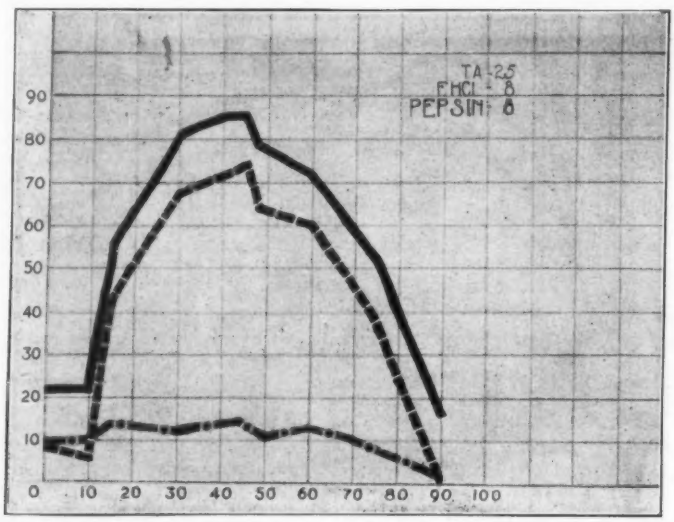

Fig. 2.—Showing a subacidity after the Ewald test and a normal secretory response of short duration after the histamine test.
——— Total acidity; - - - - - - - - free hydrochloric acid; -.-.-.-.-.-.- pepsin (mm. digested).

after at fifteen minute intervals the stomach was completely emptied of its contents, this procedure being continued until little or no secretion could be obtained. In some instances the tube was kept in the stomach for two and one-half hours or longer. At times we were compelled, because of the discomfort to the patient, to remove the tube before the secretion had ceased entirely. Occasionally fluid regurgitated through the tube before the end of the fifteen minute intervals. Such specimens were collected and recorded separately. Each sample was examined for volume, total acidity, "free" acidity, pepsin, blood, bile, and mucus. The pepsin content was estimated by the Mette method; the acidity was recorded in cubic centimeters of tenth-normal NaOH in acid.

The first twenty cases of our series were controlled with unusual care. During the course of the experiments, frequent blood pressure examinations

and pulse readings were made on all these persons. In many of them there was no demonstrable change after the subcutaneous injection of 1 c.c. of a 1:1000 solution of histamine; while in others there was a fall of only 6 to 8 mm. of mercury and an increase of 5 to 10 beats per minute in the pulse rate.

Up to the present date we have given over fifty injections of histamine without a disagreeable reaction. In the majority of the cases a flushing of the face and neck was noted. This came on in about five to ten minutes after the injection and lasted from fifteen to thirty minutes. Some of the patients complained of a mild headache and slight dizziness at about the same time, this latter symptom however being of a very transitory character.

The effects of subcutaneous injections of histamine have been interesting and in many instances quite surprising. In those persons in whom extraction after the Ewald meal revealed a normal or increased acidity, the re-

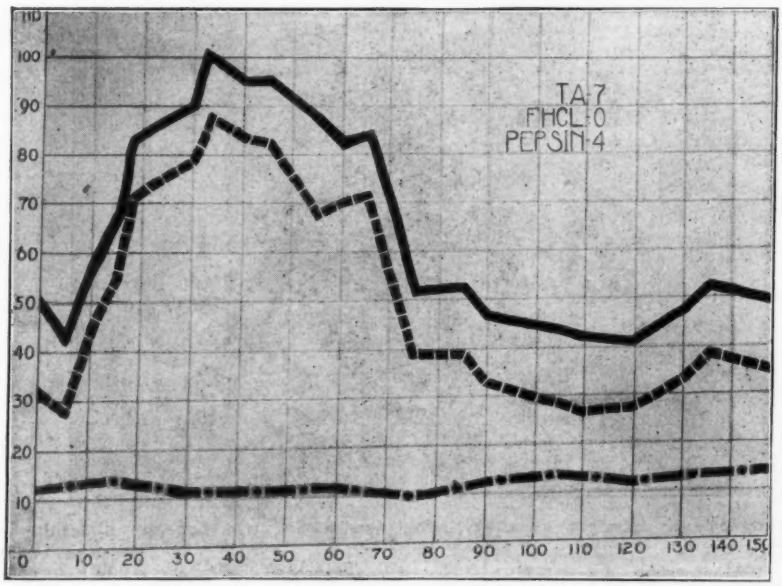

Fig. 3.—Showing an apparent anacidity after the Ewald test but the histamine response proves the secretory capacity to be within normal limits. The response is markedly prolonged. ——— Total acidity; – – – – – – – – free hydrochloric acid; –.–.–.–.–.–.– pepsin (mm. digested).

sponse to histamine also was well marked. At times the acidity attained was very high, the total acidity reaching in one instance 200 and the "free" 180. In these cases the rate of secretion was considerably accelerated, one individual secreting 135 c.c. in fifteen minutes. In fact in one instance, almost 500 c.c. of secretion was obtained in two hours and the response had not entirely subsided at the end of that time. It was, however, in the patients with subacidity or anacidity after the Ewald meal that we observed the most surprising response to the histamine test. It was impossible to foretell the response to histamine from the results of the reaction to the test meal. In some, the acidity following the injection of histamine reached the same figures as in those patients who showed a normal or increased acidity after the Ewald meal.

Our first aim in these experiments was to substantiate the work already done. This we were prepared to do in the greater part. That histamine when given subcutaneously stimulates gastric secretion is a demonstrated fact. The injection of 1 c.c. of a 1:1000 solution of histamine hydrochloride appears to be a harmless procedure.

We were not able to substantiate the statement of Matheson and Ammon that histamine produces gastric reaction in definite sequences. They maintained that, after the injection of histamine in normal cases, the peptic activity reaches its maximum in about five to fifteen minutes; whereas the total and "free" acidities reach their maxima in about twenty to twenty-five minutes, the rate of secretion reaching its maximum last in from thirty to sixty minutes.

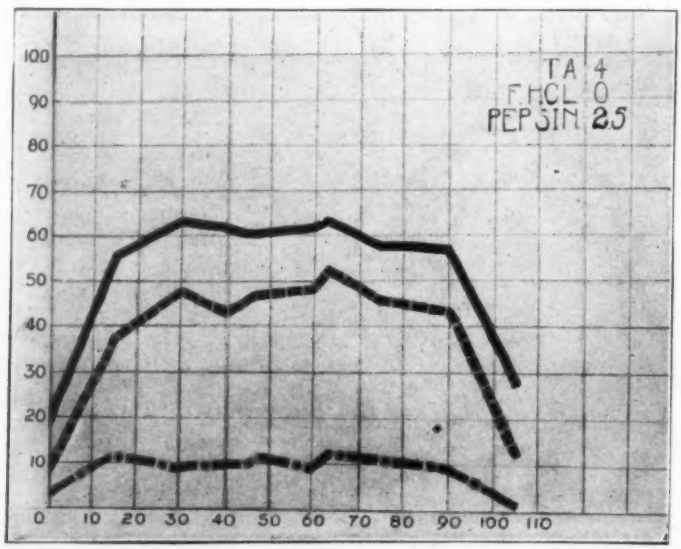

Fig. 4.—Showing an apparent anacidity after the Ewald test but the histamine response demonstrates a functional or pseudo-achylia. The secretory capacity is normal.
——— Total acidity; - - - - - - - - free hydrochloric acid; -.-.-.-.-.-.- pepsin. (mm. digested).

In our studies of abnormal cases, we were unable to determine any definite relationship between the increase in pepsin and acid production. In many patients the peptic activity reached its maximum after the acidity, in one instance the maximum of peptic activity being reached after the acidity had risen and fallen back to its fasting level.

Similarly, although 66 per cent of our cases showed their maximum rate of secretion in from thirty to sixty minutes after the injection, 22 per cent attained the maximum in from sixty to ninety minutes while 11 per cent reached it in fifteen to thirty minutes. Also in some cases the maximum of the rate of secretion was attained ten minutes before the maximum of acidity was secured. However, the differences between our results and those already reported are not very important and are perhaps explained by the fact that all our patients were the subjects of gastric disorders while the previous reports were based on studies of normal subjects.

By far the most interesting findings of our experimental studies were noted in the cases of apparent anacidity or so-called achylia gastrica. Because of this fact we shall describe our results in greater detail for this group. We included among them only such as showed a complete absence of "free" hydrochloric acid and a markedly diminished total acidity after the Ewald meal. Of seventeen cases in this group with an absence of "free" hydrochloric acid after the Ewald meal, ten or 59 per cent showed large amounts of "free" hydrochloric acid after the histamine test. The maximum amount of free acid production in these ten cases was as follows: 28, 32, 43, 46, 49, 50, 52, 57, 64 and 87 and the total acidity in each case was proportionately higher. In other words, of seventeen cases of apparent achylia gastrica, nine or more than 50 per cent produced normal or above normal acidities after the injection of histamine; while ten cases in all or

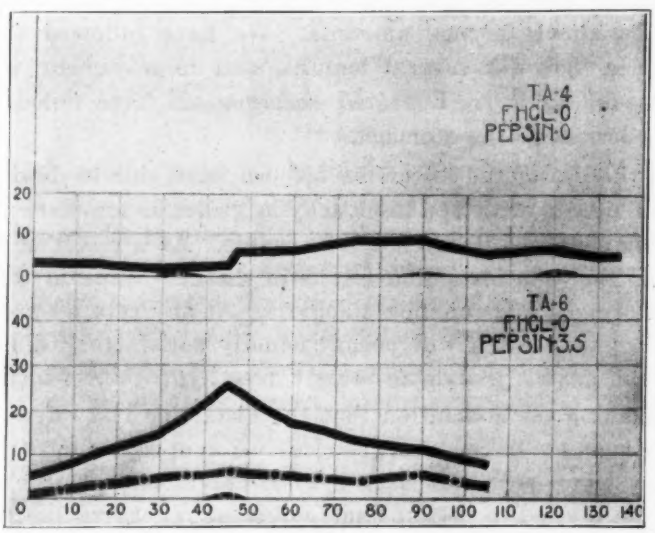

Fig. 5.—*Above.* Showing an anacidity after the Ewald test and proved by the absence of histamine response to be a case of achylia gastrica vera. *Below.* Same type of case as above except that there is a slight histamine response.
————Total acidity; - - - - - - - free hydrochloric acid; -.-.-.-.-.- pepsin (mm. digested).

59 per cent showed considerable amounts of free hydrochloric acid present. The diagnoses of these ten cases were as follows:

Alcoholic gastritis	2
Cholelithiasis	2
Chronic cholecystitis	1
Hydrops of gall bladder	1
Catarrhal jaundice	1
Carcinoma of the bile duct	1
Pulmonary tuberculosis with ptosis	1
Duodenal ulcer	1

It is therefore evident that of the seventeen assumed cases of achylia gastrica the histamine test excluded all ten, or 59 per cent. The diagnoses of the remaining seven follow:

Inoperable pyloric cancer	1
Ovarian cancer with pyloric and hepatic metastasis	1
Chronic gastritis and cholelithiasis	1
Achylia gastrica vera	4

The first three cases of this group did not at any time after the histamine injection show even a trace of free hydrochloric acid, the total acidity never rose above thirteen and pepsin was present in minimal amounts only. The last four, which we believe to represent true or organic achylia gastrica, were subjected to rigid examinations and repeated tests. These have at some time shown traces of free hydrochloric acid after histamine injections, never, however, exceeding 3 of free hydrochloric acid as a maximum figure. In one individual the total acidity rose from four to twenty-four following the histamine while the others also showed a rise in total acidity. Pepsin was present in almost normal amounts. We have followed these cases of achylia gastrica vera for several months, and improvement was noted on hydrochloric acid therapy. Repeated examinations have failed to show any demonstrable lesion of the stomach.

Thus by the use of the histamine test we were able to determine that of seventeen individuals with apparent achylia gastrica, ten were pseudo, functional or incomplete, while seven were organic. As a result of these experiments we feel that the histamine test is of distinct value in the differentiation between organic and functional achylia. At any rate we are not familiar with any other test which will permit equally satisfactory diagnosis of the capacity of the gastric glands to secrete free hydrochloric acid.

After reaching the conclusion that the histamine test offered a means of differential diagnosis in achylia gastrica, we centered our attention on its possible effect upon cases of early pyloric carcinoma. Our experience has as yet been too limited to permit final judgment regarding the possible value of the histamine test in this condition. In individuals suffering from carcinoma of the stomach the reaction has shown a very small increase, if any, over the Ewald figures. In no other gastric conditions investigated has the histamine response shown such a small increase over the Ewald figures. In view of these findings we believe that probably the carcinomatous patients were suffering from a disease which was destroying the secreting glands.

Whether, as Matheson and Ammon propose, histamine may be of use as a therapeutic agent in this field, remains to be demonstrated. Possibilities of its usefulness are suggested in cases of inanition due to tuberculosis, nervous shock or any condition where the discharge of hydrochloric acid and pepsin is inhibited. Histamine given subcutaneously immediately after a meal may promote secretion and consequently gastric digestion. Such indicated possibilities of usefulness deserve a clinical trial.

CONCLUSIONS

1. Histamine when injected subcutaneously into human subjects produces a stimulation of the flow of gastric secretion.

2. The subcutaneous injection of 1 c.c. of a 1:1000 solution of histamine hydrochloride is apparently harmless.

3. The histamine test is of value in the differentiation of true and pseudo achylia.

4. The histamine test *may* be of assistance in the early diagnosis of pyloric carcinoma.

5. Histamine offers possibilities as a therapeutic agent in conditions of diminished digestive function.

A COMPARISON OF THE INTRADERMAL AND SUBCUTANEOUS INJECTIONS OF INSULIN IN THE PRESENCE OF SUPRARENIN*

By E. F. Müller, M.D., and H. B. Corbitt, Ph.D., New York

THE action of insulin varies according to the tissues into which it is injected. This phenomenon has been discussed in previous publications[1, 2, 3] and the attempts made to explain it have given rise to many more questions related to the mechanism of the action of insulin.

It has been demonstrated that in normal rabbits the hypoglycemic action of a given quantity of insulin is dependent upon the route of administration. Intravenously, the action is early and of brief duration; given subcutaneously, the initial lowering occurs later and is more prolonged; given intradermally, the initial decrease approximates that observed after subcutaneous injection but its duration is much longer and the figures are lower both four and six hours after injection. Thus, it is shown that the further from the blood stream the insulin is injected, the more effective and prolonged is the influence exercised on the blood-sugar content. This was at first assumed to be due to a difference in the rate of absorption of the specific blood-sugar lowering substance. However, a study of the blood-sugar curves observed after different methods of administration, i.e., after injection into different body tissues reveals the fact that differences in the rate of absorption do not explain the resulting differences in the blood-sugar curves.

In order to obtain additional information which might be used in solving the problem of the mechanism of the action of certain drugs and its relation to the mode of administration, further studies were made in this field. These experiments involved a study of the action of insulin under the influence of simultaneous injections of suprarenin (synthetic epinephrin).

It is known that suprarenin injections increase the blood-sugar content but it should not be concluded that this action of suprarenin always tends to neutralize the action of insulin. On the contrary, as shown in a preliminary report,[4] suprarenin in some instances actually increases the action of insulin

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when given simultaneous with or previous to the usual subcutaneous injection of insulin. The following results are presented as further illustration of the action of insulin given intradermally in the presence of suprarenin, to show whether or not it acts still differently from the subcutaneous administration.

Normal rabbits were kept under conditions described in our previous publications. The animals were starved for about twenty-two hours prior to use. After determining the normal blood sugar an injection of 0.05 mg. suprarenin per kilo body weight was made subcutaneously; thereupon the insulin was immediately injected at a different site, either intradermally or subcutaneously. If the suprarenin was injected over the right hind leg, the insulin was injected over the left fore leg. The results are given in Table I.

TABLE I

SUBCUTANEOUS INJECTION OF 0.05 MG. SUPRARENIN PER KILO BODY WEIGHT WITH A SIMULTANEOUS INJECTION OF 0.5 UNITS OF INSULIN PER KILO BODY WEIGHT. PERCENTAGE OF CHANGE IN SUGAR CONTENT.

RABBIT	INSULIN SUBCUTANEOUSLY			INSULIN INTRADERMALLY		
	Hours after Injection			Hours after Injection		
	2	4	6	2	4	6
58	−33.1	−24.8	+ 5.0	−33.6	−46.8	−41.1
76	−35.6	+28.8	+38.6	−22.2	−16.3	+ 2.6
				−27.5	−26.7	− 5.8
78	−26.8	−41.3	−42.3	−46.3	− 5.7	− 0.8
79	−35.6	−37.6	−13.7	−44.8	−52.8	−37.6
84	+13.8	+41.3	+28.8	+14.8	−28.2	−23.4
85	+26.5	+ 6.0	+ 2.6	+61.6	− 4.5	− 0.9

It is noted that in four of the six rabbits the lowering of the blood sugar at four and six hours is more marked after intradermal injection than when the subcutaneous route of administration was employed. With some rabbits the decrease due to intradermal injection of insulin seems to have been affected. One rabbit (No. 85) showed the same figures in both instances; another (No. 78) gave a more marked reaction after a subcutaneous injection of insulin combined with suprarenin than after insulin alone, given either subcutaneously or intradermally. This reaction seems to be peculiar to this rabbit, as will be seen upon reference to Table II. Eadie and Macleod[5] have recorded a similar peculiar reaction with one of their animals. They used a much larger dose of suprarenin, as well as a larger dose of insulin.

Table II presents the results of the injection of insulin and suprarenin given simultaneously and at the same site. The solutions of the two drugs were mixed in such proportions that the final solution insured the administration of 0.05 mg. suprarenin and 0.5 units of insulin per kilo body weight respectively.

In this series of experiments wherein the substances were injected in one solution the action was observed to differ from that following a separate injection of the drugs at different sites. In Rabbits 58 and 76 the action of insulin is neutralized by the suprarenin content of the solution, following both the subcutaneous and the intradermal administration. The same applies to Rabbits 78 and 82 in the case of intradermal injection. In Rabbits

TABLE II

THE INJECTION OF MIXED SOLUTIONS OF SUPRARENIN AND INSULIN IN DOSES OF 0.05 MG. AND 0.5 UNITS PER KILO BODY WEIGHT RESPECTIVELY. PERCENTAGE OF CHANGE IN SUGAR CONTENT.

RABBIT	SUBCUTANEOUS			INTRADERMAL		
	Hours after Injection			Hours after Injection		
	2	4	6	2	4	6
58	+12.5	−20.8	− 6.3	− 0.9	− 9.5	− 0.9
76	− 6.0	− 8.6	− 4.3	+11.6	− 6.3	− 9.0
				− 1.6	− 3.2	− 1.6
78	−45.1	−68.5	−41.3	− 7.1	−11.8	− 3.2
79	+ 1.6	−37.3	−32.6	−25.8	−33.5	−29.8
80	−50.5	−53.3	− 3.3	−36.0	−40.8	−12.8
				+ 9.2	+22.0	+30.3
82	−53.4	−14.3	+ 2.5	+12.9	− 7.6	− 1.5

78, 80, and 82 the action of insulin was more pronounced than when the drug was injected alone by the subcutaneous route. In Rabbit 79 the results were practically identical with those after the injection of insulin alone intradermally. A further examination of the results presented in Table II discloses that the introduction of a new factor, the intradermal injection of suprarenin, has given rise to a different action than when the drug was injected subcutaneously. Because of this new action it was deemed advisable to continue these studies, giving suprarenin intradermally and at a site well removed from that used for the injection of insulin. The results of these experiments will be presented in the near future.

A consideration of the experiments given in Table I allows but one conclusion. It had previously been determined by us that insulin administered intradermally was more effective than when the subcutaneous method was employed. A confirmation of these findings will be noted in the present observation in that even when given in the presence of suprarenin, insulin is more effective by intradermal methods than by the subcutaneous route.

This confirms our earlier results and establishes from another point of view the theory of special properties of the skin, the nature of which still remains unknown as far as the action of specific drugs is concerned.

The results of the experiments with mixed solutions of insulin and suprarenin have as yet found no satisfactory explanation owing to the unknown action of intradermal injections of suprarenin.

SUMMARY

1. Insulin, injected in rabbits at a different site simultaneously with injections of suprarenin, proves more effective by the intradermal than by the subcutaneous route.

2. In rabbits receiving the two substances in one solution the greater insulin effect is noted when the subcutaneous method of administration is employed, in those cases which do not show a mutual neutralization.

3. If both drugs are injected intradermally in the same solution their respective actions are partly neutralized.

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THE MECHANISM OF THE PRIMARY FALL OF BLOOD PRESSURE FOLLOWING EPINEPHRIN INJECTIONS*

By George R. Love, M.D., Urbana, Illinois

In dogs, the intravenous injection of one cubic centimeter or more of 1 : 10,000 solution of epinephrin usually produces a blood pressure curve characterized by an abrupt primary rise, an irregular primary fall, a secondary rise, and a gradual secondary fall to, or below, the original level. The purpose of this investigation was to study the mechanism involved in the primary fall, which follows the primary elevation of blood pressure.

Oliver and Schafer[1] state that the inhibition of the intact mammalian heart following the primary rise of blood pressure by epinephrin is of central vagal origin. Elliott[2] states that inhibition is most manifest in animals whose vagi are intact; nevertheless, section of the vagi in the neck does not entirely remove the tendency of the heart to be slowed and to occasionally drop a beat. Under the strain of the high pressure, a peripheral nervous reflex, presumably of the axon type, is originated, which tends to overpower the accelerating influence of adrenalin.

McGuigan and Hyatt[3] made a special investigation of the primary depression and the secondary rise in blood pressure caused by epinephrin. They suggest that the primary fall, if uninfluenced by central action, would continue to fall; however, blood pressure changes within the brain or connected with the circulation of the brain, change the primary fall so that in many cases a secondary rise occurs. They conclude that the cause of the secondary rise is apparently due to a central action of epinephrin (indirect), the efferent impulse being conveyed through the sympathetic ganglia. The basis for this belief was that the phenomenon occurred after section of the vagi or after atropine, but was prevented by ablation of the head, pithing of the brain, high section of the cord, and paralysis of the sympathetic ganglia with nicotine.

In view of the fact that reports of previous investigations are somewhat conflicting, a more thorough investigation seems justified.

*From the Department of Pharmacology and Therapeutics, University, of Illinois, College of Medicine, Urbana, Ill.
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METHODS

All experiments were made upon dogs under light ether anesthesia by tracheotomy. Chloroform was substituted in many of the experiments for comparison. Blood pressure was recorded either by the mercury or membrane manometer. The movements of the heart were recorded by the Cushny myocardiograph, the adjustable tambour or lever methods.[4] Ventricular volume was recorded by the rubber diaphragm cardiometer with air conduction to a large tambour or water manometer. Freshly prepared 1 : 10,000 solution of adrenalin was used in all experiments.

PERIPHERAL RESISTANCE

That the primary fall might be due to a decreased peripheral resistance is suggested by the fact that epinephrin has a vasodilator action.[5, 6, 7, 8, 9] The hypothesis suggested by McGuigan and Hyatt also involves the peripheral resistance.

I have been unable to demonstrate changes in the peripheral resistance by plethysmograph methods which would account for the primary fall. The vessels of some tissues may undergo primary dilatation, while others are constricting;[1, 10] however, this primary dilatation occurs during the primary rise of carotid blood pressure, and is usually supplanted by constriction at the beginning of the primary fall.

The constricting action of epinephrin progresses rapidly, and is most marked during or near the peak of the secondary rise. Plethysmograph tracings of the abdominal viscera, extremities, nasal, and buccal membranes (technic[11]), show a decrease of volume which begins during the primary rise and is greatest near the summit of the secondary rise. The volume curves give no evidence of a decreased peripheral resistance during the primary fall. Also, the blood pressure curves after epinephrin often show various fluctuations which are not related to the peripheral volume curves. The pulse pressure (membrane and mercury manometers) on the primary fall, when unaccompanied by marked inhibition of rate, is decreased. This is not indicative of decreased peripheral resistance.

Hartman and Frazer[8] give evidence that epinephrin has a central vasodilator action upon the limbs and intestines. Erlanger and Gasser,[12] however, have shown that after prolonged epinephrin administration, the constriction of the somatic and splanchnic areas is maximal and in part of central origin.

THE HEART

The changes in the heart accompanying the epinephrin blood pressure curves are not constant. However, in ninety-seven experiments performed, the primary fall, when it occurred, was in the majority of cases of cardiac origin. The cardiac behavior during the primary fall is not constant in different dogs, and not uncommonly of a different character in the same dog upon subsequent injections. A few changes occurred so infrequently that the mechanisms could not be studied satisfactorily, and are subject to reconsideration. The primary fall does not, therefore, represent a definite mechanism.

We have assumed from the results of other investigators,[2, 13, 14, 15, 16] that the caval pressure, if not increased, is at least effective even after massive doses of epinephrin.[12]

THE VAGI

In a majority of adult dogs, one cubic centimeter of 1 : 10,000 epinephrin solution injected intravenously produces (after the primary rise) an abrupt primary fall of blood pressure, which is due chiefly to central vagus stimulation. (See Figs. 1 and 2.) In some dogs, especially pups, epinephrin injections in doses from 1 to 30 c.c. failed to elicit a vagus stimulation or a primary fall, although the blood pressure rose unusually high. It is known that in very young animals the vagus is sometimes inactive.

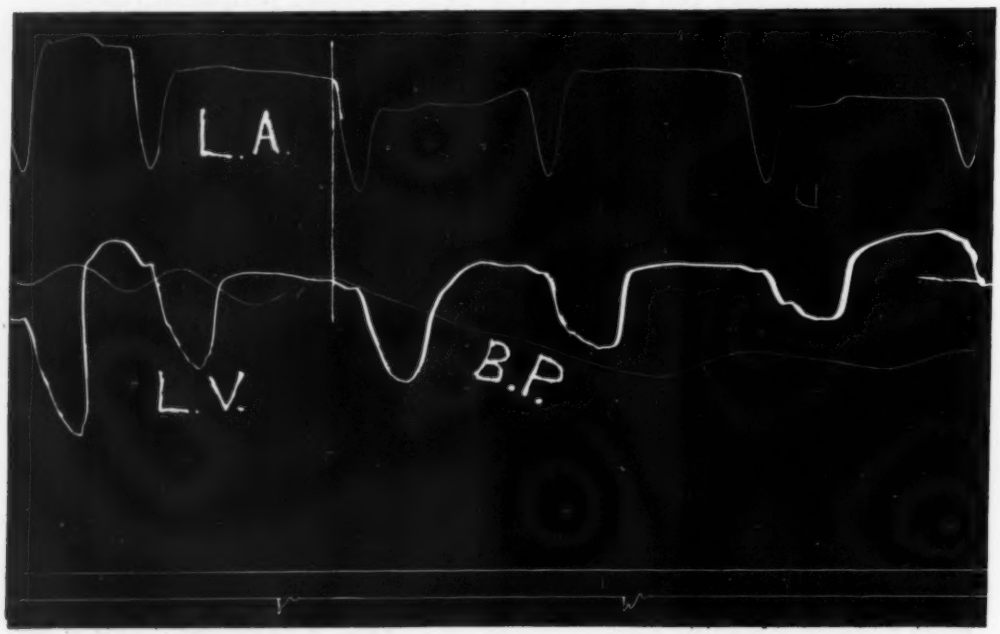


Fig. 1.—Left auricle and ventricle, systole=downstroke, spring levers. Time in seconds. Epinephrin 0.1 mg.: dog, 7 kilo. Peak of the primary rise and onset of the primary fall due to central vagus stimulation. For proof see text.

The proof that vagal stimulation is mainly responsible for a primary fall is based on the fact that after obtaining marked inhibition during the primary fall once or several times, section of the vagi in the neck, or 2 mg. atropine intravenously, change the entire contour of the blood pressure curve upon subsequent injections of the same dose of epinephrin, and in over 50 per cent of cases, eliminate the primary fall entirely. The blood pressure curve now describes a simple rise and fall.

The vagal action may be one of several types of sinus arrhythmia, as follows: (1) An inhibition of regular rate. (2) Coupled beats in which the weaker beat occurs after a longer pause; its volume ejection is less complete than the stronger beat which follows it after a shorter pause. The second beat appears to break through the vagal inhibition. (3) Phasic sinus arrhythmia of a more irregular character occurs more frequently from larger doses

of epinephrin (2 to 3 c.c.), but sometimes is quite distinct following a 1 c.c. injection. Sinus arrhythmia may produce several fluctuations following the primary rise, and in many cases a typical secondary rise does not occur. Numerous variations have been noted from simple predominating vagal inhibition to extreme fluctuations of vagal and accelerator activity. The larger doses tend to stimulate the accelerators sufficiently to break through the vagal influence more often, and as a result, several fluctuations occur. The mechanism of each succeeding rise and fall is usually identical with its primary rise and fall until blood pressure falls to normal or below.

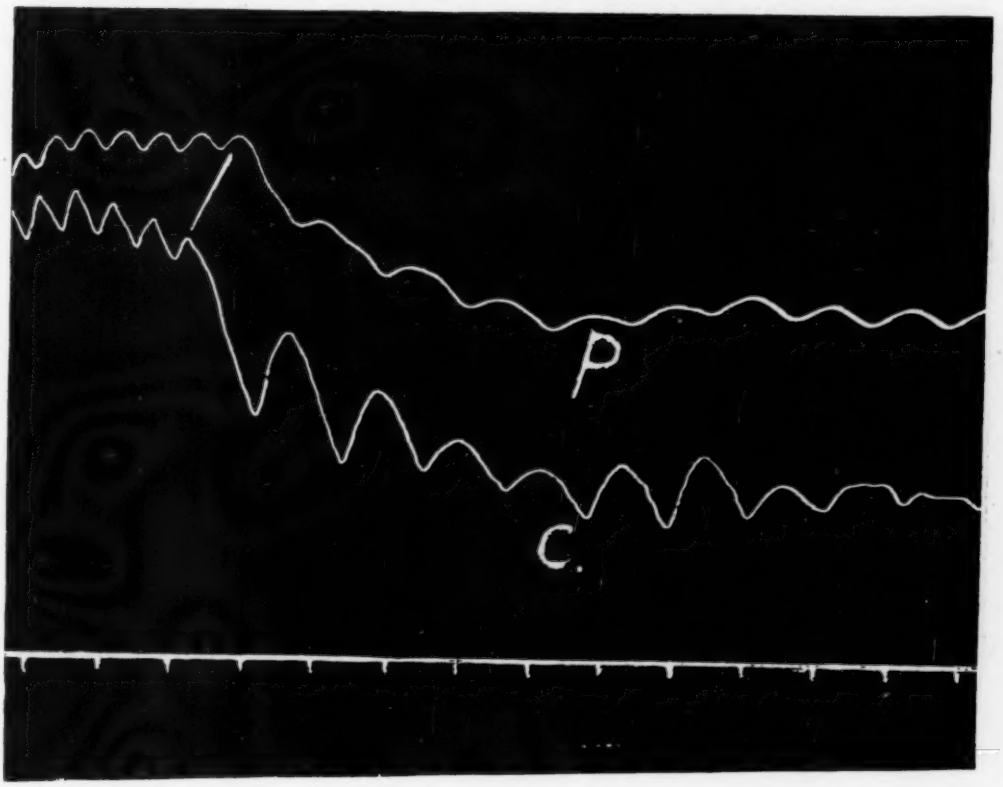


Fig. 2.—Pulmonary and carotid blood pressures. Time in 0.5 seconds. Peak of primary rise and onset of primary fall due chiefly to central vagus stimulation. For proof see test.

Vagal inhibition does not always cause a primary fall. This is evident if the slowing occurs early on the primary rise, if the onset is gradual, or if the inhibition is not marked. Apparently, in such cases the increased systolic discharge is sufficient to maintain the blood pressure.

The changes described hereafter occur also with the vagi severed.

ATRIOVENTRICULAR BLOCK

Atrioventricular block may occur after section of the vagi or atropine. It is usually of the irregular or 2-1 types, and in some cases is the cause of a primary fall. (See Fig. 3.) The onset of the block may or may not inaugurate the primary fall. In the latter case, the blood pressure begins to fall

for several beats preceding the block. The only constant change we have observed in the heart, preceding the block, is a decrease of the systolic ejection gradient. Incomplete block occurs more often if chloroform is administered. The "delayed inhibition" described by Oliver and Schafer[1] and the very marked inhibition following adrenalin (chloroform anesthesia) reported by Heinekamp[17] have been in my experiments not sequential inhibition, but incomplete atrioventricular block. The immediate effect of the block is a marked fall of pressure. (See Fig. 3.) As the systolic discharge increases, the pressure soon begins a slow rise, and with the return of normal sequence, becomes quite abrupt. The blood pressure picture varies with the duration of the block, which may be from a few seconds to a few moments.

The larger doses of epinephrin may produce an apparent disassociation of auricles and ventricles without bradycardia. The disassociation, or at least the auricular filling function, is often intermittent, and, likewise, produces

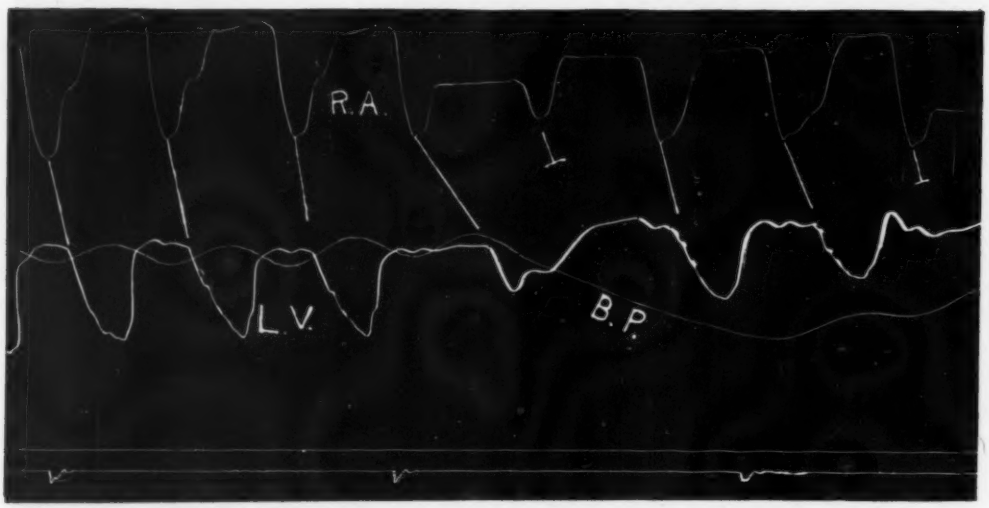


Fig. 3.—Right auricle and left ventricle, spring levers, systole=downstroke. Time in seconds. Dog, 9 kilo: vagi severed: epinephrin 0.15 mg. Peak of primary rise and onset of primary fall due to irregular atrioventricular block. A few beats later this became established as a 2-1 block, which continued for about thirty seconds.

fluctuations of blood pressure. The secondary rise occurs with the return of normal sequence. Kahn[18] reported complete heart block following epinephrin injection, without bradycardia. Epinephrin, it is said, also causes acceleration of both auricles and ventricles after experimental block.[19, 20, 21]

PREMATURE CONTRACTIONS

Premature contractions of ventricular origin may contribute to or cause a primary fall. They may occur as isolated contractures, bigeminy, trigeminy, or in a series which predominates the rhythm. The isolated premature contractions are common with ether anesthesia, whereas under chloroform anesthesia the complexes also frequently appear. Such irregularities have been described also by Levy and Lewis.[22, 23]

Premature auricular contractions often occur after epinephrin, but are not in themselves a specific cause for a primary fall of pressure.

VARIATIONS OF CONTRACTION PROCESS

A hypodynamic heart (chloroform or exposure) which responds to epinephrin, shows at first a decrease of volume from the cardiac acceleration. However, as the blood pressure rises, dilatation again becomes evident. A heart in good condition shows very little change in volume until the blood pressure rises considerably, at which time there may be a variable change in volume. A common occurrence at the peak of the primary rise is the development of an apparent depression of contractile power, which is a frequent cause for a primary fall. This temporary insufficiency may be manifested in several ways, as (1) pulsus alternans, (2) incomplete systole and diastole, (3) incomplete systolic effort with acute dilatation.

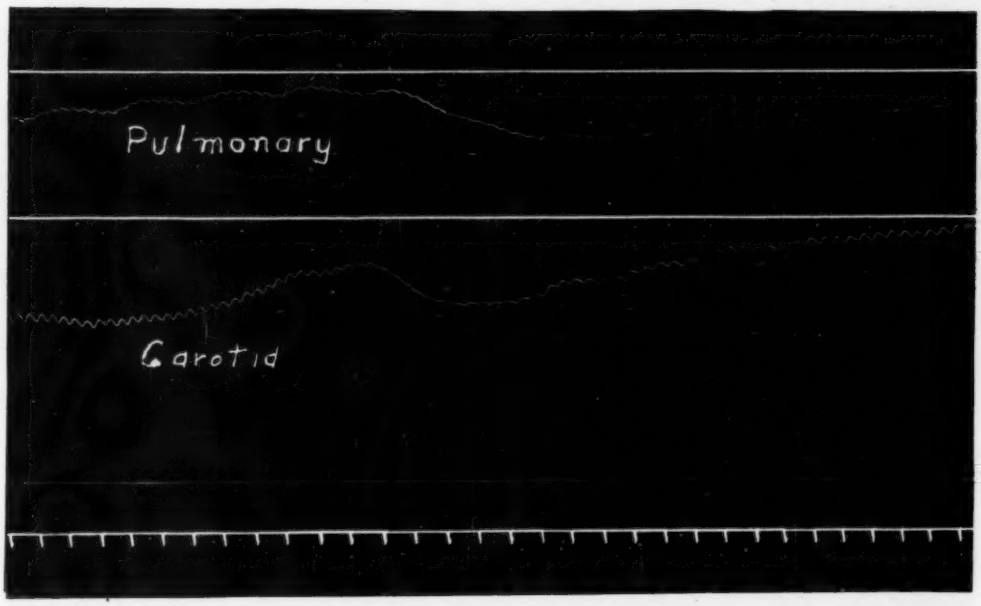


Fig. 4.—Pulmonary carotid blood pressures, time in seconds. Dog, 6 kilo: vagi cut. Primary rise and fall and secondary rise following 0.1 mg. epinephrin. The synchronous primary fall of pulmonary and carotid pressures, together with a decrease of pulse pressure, indicates that the mechanism is of cardiac origin and not a peripheral change.

Pulsus Alternans.—Pulsus alternans may be a ventricular alternation (more often in hypodynamic hearts) or a concordant atrioventricular alternation. Stimulation of the accelerator nerves is reported to produce pulsus alternans.[24] The changes in rate during alternation are often marked, but their direction is variable. The criteria for the primary fall and secondary rise in such cases depend ultimately upon the steepness and completeness of the systolic volume curves. In concordant atrioventricular alternation, the weaker beat may pass to a pulsus deficit and occasionally drop from the cycle. In one instance there was a sudden permanent omission of the weaker, but still effective, beat, causing an instant halving of A - V rate.

Incomplete Systole and Diastole.—This condition is often preceded by a short period of alternating or irregular beats, after which every beat becomes weak with usually, but not always, an acceleration of rate. The ven-

tricles work from a position of semisystole, i.e., they neither relax nor contract completely. This is evident from volume curves and direct inspection. (See Fig. 6.) Lever methods are entirely inadequate and their records unintelligible in this condition. The heart passes back through the stage of weak or alternating beats to equal strokes on the secondary rise.

The failure of the ventricles to relax and fill properly indicates excessive tonus, for the auricles are full and their systole is apparent upon the ventricular volume curve (Figs. 5, 6). That the condition is not primarily due to

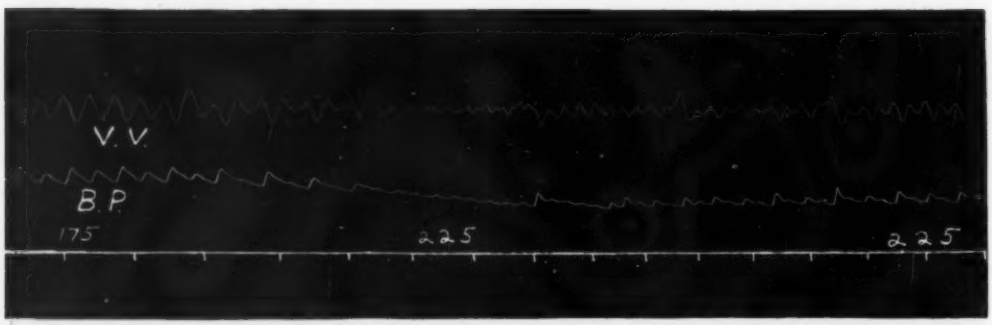


Fig. 5.—Ventricular volume and carotid blood pressure by membrane manometer, time in seconds. Dog, 9 kilo: vagi cut: epinephrin 0.15 mg., fourth dose. The beginning of the primary fall is due to an alternate pulse. The pressure falls farther as every stroke becomes weaker, and the ventricles fail to fill or contract effectively. Other irregularities, not apparent from this single tracing, occur as the secondary rise begins. The pulse becomes alternate again and later equal as the secondary rise progresses. The periodic changes on the volume curve—about every 3 seconds—are due to artificial respiration. The acceleration of rate appears secondary. (See Fig. 6.)

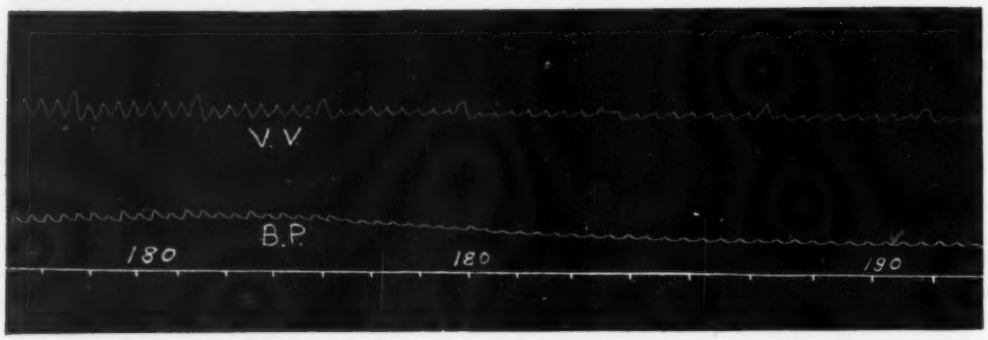


Fig. 6.—Repetition of same dose one hour later than Fig. 5. The inability of the heart to accelerate distinctly during the fall, indicates that in Fig. 5 the acceleration is, at least, not an essential factor. The ventricles are in a position of semisystole or tetany: they neither relax nor contract effectively. The periodic variations on the volume curve, about every 3 seconds, are due to artificial respiration.

increase of rate is evidenced by the fact that the ventricular movements become effective to produce the secondary rise, and often with a further increase of rate.

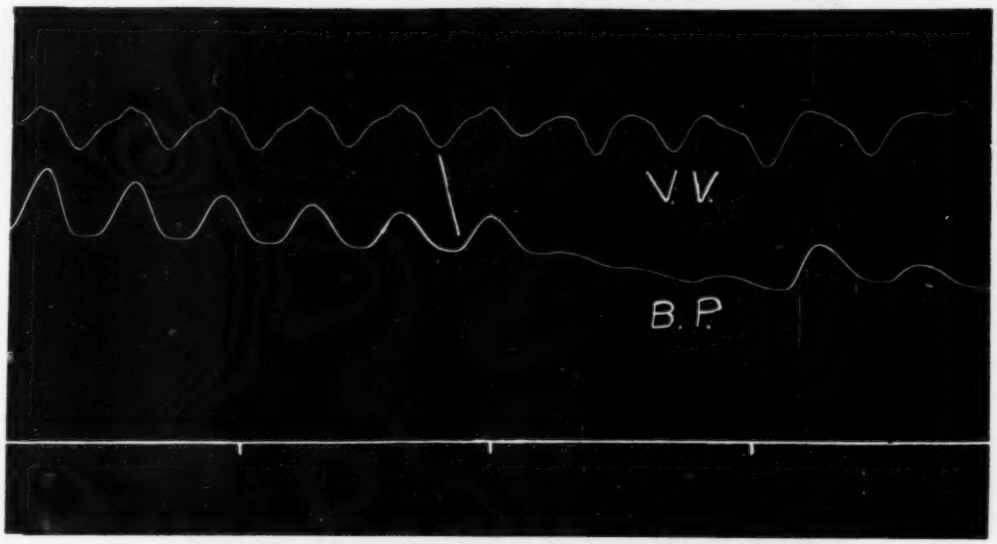
DILATATION

Acute dilatation, instead of incomplete relaxation, often accompanies the ineffective systolic effort (see Fig. 8). This condition is due to dilatation of the left ventricle, because carotid pressure falls before the pulmonary pressure. The pulmonary pressure falls after dilatation is well marked, prob-

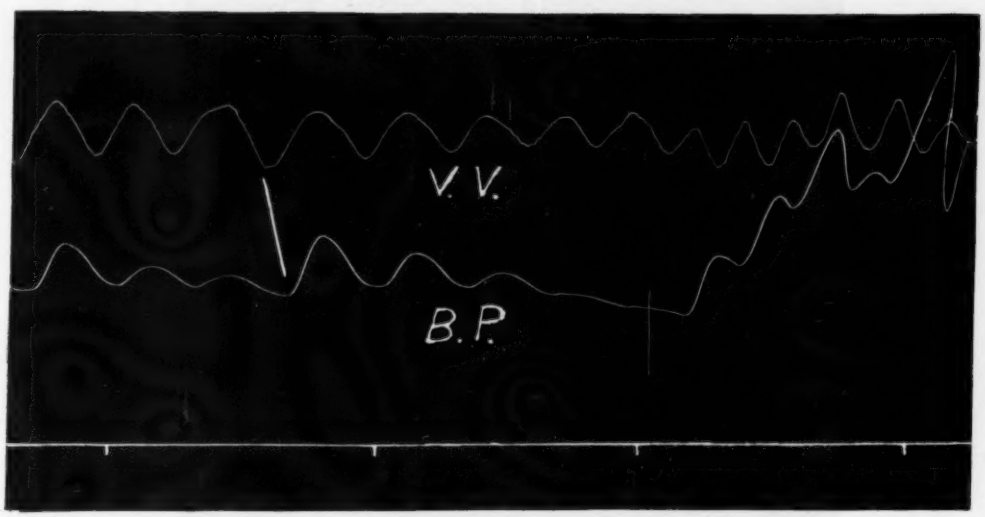
ably from a reduction of filling capacity of the right ventricle, due to the protrusion of the interventricular septum. Acute dilatation occurs more frequently in hypodynamic hearts.

DISCUSSION

As stated previously, the primary fall and secondary rise do not represent a definite mechanism. Likewise, there is no one typical blood pressure curve. The ultimate result represents temporary changes of output, which



A.



B.

Fig. 7.—Ventricular volume and blood pressure by membrane manometer. Dog, 9 kilo: vagi cut: epinephrin, 0.15 mg., previously.

A.—The peak of primary rise and beginning of a primary fall. Note gradual decrease of pulse pressure, followed by irregularities of rate, rhythm, and force.

B.—The beginning of a secondary rise. Note rapid filling and increased velocity of ejection, with a marked increase of rate.

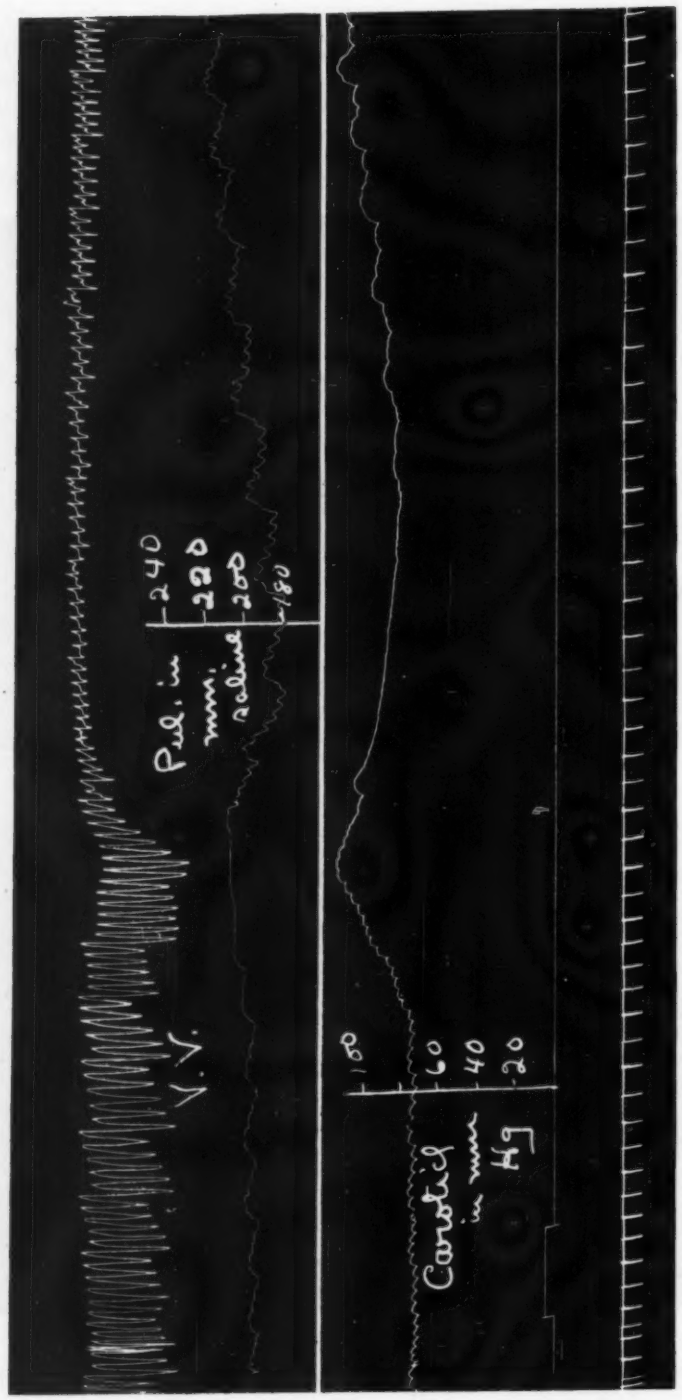


Fig. 8.—Ventricular volume, pulmonary and carotid pressures, time in seconds. Dog, 7 kilo; vagi severed; injection of 0.1 mg. epinephrin. Primary fall due to inadequate systolic effort accompanied by acute dilatation.

are accomplished by alterations in rate, rhythm, and force of the heart beat. The fact that the primary fall is variable and not constant, and also that several factors may operate simultaneously, make the interpretation of the records extremely difficult. Undoubtedly some alterations have passed by unnoticed.

The conditions described as occurring after section of the vagi are attributed to three factors, namely, (1) accelerator stimulation, (2) increased irritability of the heart muscle, and (3) aortic pressure. In ventricular alternation and acute dilatation, the aortic pressure is more important; however, alternation would probably be less prominent if the rate was unchanged during the primary rise. The time relation of the primary fall includes the period of most intense action on the heart from a single injection of epinephrin.

The absence of the primary fall upon elimination of the central nervous system may be explained in part from the observations of Levy,[22] who found that the tendency for cardiac irregularities was diminished under such circumstances.

That obstruction in the pulmonary circuit might cause the primary fall was eliminated in many experiments by simultaneously recording pulmonary arterial pressure (Fig. 4). Schafer and Lim[25] found that the rise of pulmonary pressure on dogs and cats following epinephrin was chiefly of cardiac origin. These authors also observed fluctuations of both pulmonary and carotid pressures, which they attributed to cardiac irregularities. Wiggers[26, 27] found that during the rise of pulmonary pressure after epinephrin, the actual flow in the lesser circuit, as determined by the flow from a small pulmonary vein, was increased.

That epinephrin, or the high blood pressure produced by its injection, may cause a sequential inhibition of rate (vagi severed) seems well established.[2, 15, 16, 17] However, I do not believe the changes are ordinarily so abrupt that the augmented discharge cannot maintain the systolic pressure.

Epinephrin does not seem to have a specific depressing action upon the conduction system. In a personal communication, Carl J. Wiggers expressed an opinion from his own observations that the block produced by epinephrin after section of the vagi was due to so great an acceleration of the auricles that not every impulse is conducted. My experiments tend to confirm this opinion. The A - V intervals in hearts in which incomplete block was produced was abridged from 10 to 30 per cent over normal, following epinephrin until the onset of the block. The A - V interval of hearts which did not develop a block was also shortened following epinephrin. Clinically also, epinephrin appears to improve conduction.[28]

CONCLUSIONS

1. The intravenous injection of 0.1 to 0.3 mg. of epinephrin in dogs produces a blood pressure curve, characterized usually by a primary rise, a primary fall, a secondary rise, and a secondary fall.

2. The primary fall is not due entirely to one definite mechanism, but is usually due to a temporary decrease of minute output in which one or more of the following mechanisms may be operative:

(a) central vagus stimulation,

(b) atrioventricular block,

(c) premature contractions,

(d) pulsus alternans and allied mechanisms representing defective contractile function, and often associated with either

(e) defective filling from abnormal tonus, probably due in part to accelerator stimulation, or

(f) acute dilatation from high aortic pressure.

3. The latter conditions, (b), (c), (d), (e), and (f), may occur also after section of the vagi, but are factors of less importance than the central action.

I wish to acknowledge my indebtedness to Hugh McGuigan for suggestions and criticism of this work and to Dr. Wiggers for information concerning epinephrin block.

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THE RELATION WHICH VARIATION IN SUSCEPTIBILITY AMONG GUINEA PIGS BEARS TO THE ACCURACY OF VIRULENCE TESTS*

By Ruth Gilbert, A.M., M.D., and H. H. Owen, A.B., Albany, N. Y.

IT has been suggested by various workers that guinea pigs may occasionally possess some natural immunity to diphtheria toxin.

Theobald Smith,[1] in 1904, stated that variations in susceptibility were the rule rather than the exception and that this condition constituted the most uncertain element upon which toxin-antitoxin tests on guinea pigs depend. He observed that dark guinea pigs were relatively less susceptible than light-colored ones.

Nuttall and Graham Smith[2] mentioned that though variation in susceptibility is not of much importance, it should, nevertheless, be borne in mind as a possible source of error in determining the degree of virulence of diphtheria strains.

Smeeton,[3] in 1917, found that variation in susceptibility of the guinea pigs used exerted a slight influence on the results of her tests. In two instances, animals inoculated subcutaneously showed no reaction, while those inoculated intracutaneously showed marked necrosis. Repeated subcutaneous tests proved the strains to be virulent and the failure of the first tests was probably due to natural immunity of the animals.

Jordan, Smith and Kingsbury,[4] in 1922, reported variations in the reactions to subcutaneous inoculations with virulent strains. They stated that since all the broth cultures used showed good growth, it was evident that some of the guinea pigs employed possessed natural immunity. They also observed that the surviving animals were usually dark-colored.

The study in this laboratory was undertaken to determine whether or not there is sufficient variation in the susceptibility of guinea pigs to prove a source of error in the performance of virulence tests.

According to the routine procedure each strain isolated, which has the typical morphological and cultural characteristics of diphtheria bacilli, is injected intradermally into two guinea pigs, one normal and one immunized with 500 units of diphtheria antitoxin. Four strains are tested on one pair of animals. Each strain which fails to induce a characteristic lesion on the normal guinea pig is injected subcutaneously into another normal animal. In case the guinea pig survives this inoculation and is perfectly well at the end of five days, it is discharged and the strain tested is reported nonvirulent.

For seven months (September, 1923-March, 1924) the susceptibility of each guinea pig which failed to give a definite reaction was tested. During

*From the Division of Laboratories and Research, New York State Department of Health, Albany, Augustus Wadsworth, M.D., Director.
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this time there were approximately ninety guinea pigs used for the intradermal test. It was necessary to determine the susceptibility of only two of these animals. (On each of the others at least one of the cultures tested gave a definite reaction.) On one of these guinea pigs all four strains tested gave no reaction. Three of these cultures proved virulent when repeated tests were made, both by the subcutaneous and intracutaneous method. The guinea pig which had shown the four negative reactions was tested later with a culture of diphtheria bacilli known to be virulent and only a very weak reaction was obtained. On the other guinea pig, the susceptibility of which was questioned, two strains induced no reaction and two others a weak reaction. Both the latter proved virulent when further tests were made. When this animal was tested with a culture known to be virulent, a weak reaction was again obtained.

Although the possibility of faulty technic cannot be excluded in interpreting these results, it seems probable that these two guinea pigs possessed some degree of immunity.

During the seven months, eighty-five cultures which gave no reaction by the intradermal method were also nonvirulent when tested by the subcutaneous method. The susceptibility of the eighty-five animals used for these subcutaneous inoculations was tested by injecting them with a strain of *B. diphtheriae* known to be virulent. Within two or three days all of these animals died showing the typical lesions produced by diphtheria toxin.

CONCLUSION

Since only two guinea pigs among approximately 175 tested, seemed to show a slight degree of immunity to diphtheria toxin, from a practical viewpoint the variation in susceptibility of these animals does not appear to constitute a source of error in testing the virulence of *B. diphtheriae* according to the procedure described.

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THE MUTUAL RELATIONS EXISTING BETWEEN THE CLINIC AND THE LABORATORY*

By Bowman Corning Crowell, M.D., Philadelphia, Pa.

THE subject on which I have been asked to speak to you this evening is the mutual relations that should exist between the pathologist and the practitioner of medicine. If my remarks are made from the standpoint of the laboratory man, it is because I am best qualified to speak from this viewpoint. I trust, however, that I may be able to emphasize certain phases of the subject that will appeal to the mind of the practitioner, and perhaps aid the worker in deriving the maximum amount of benefit from the laboratory. My remarks will have especial reference to the hospital laboratory, and I may add that, in my opinion, it is essential that relations similar to those that I shall indicate should exist between the practitioner and the so-called private or commercial laboratory if the latter is to perform its function properly.

In the first place, the man in charge of the work in the hospital laboratory today is a development of recent times. He is required to solve problems of many kinds, morphologic, chemical, and humoral, and he must have at his command an insight into many branches of the medical sciences. His primary requisite, however, is a thorough knowledge of the technical methods that may be applied for the detection of diseased conditions. In so far as he is purely a laboratory worker he deals not with the patient, but with the individual elements that go to make up the patient. To him come the blood, urine, feces, gastric contents, spinal fluid, exudates, transudates, or tissues from the patient, but not the patient himself. In order best to perform his proper function he should be able to make the requisite examinations and to indicate the influence, on the patient, of the abnormal conditions that he encounters in his study of the material submitted for examination. There is no need for him to see the patient or to have a knowledge of practical medicine in order to determine that a Wassermann reaction is positive, that the red cells are diminished in number, or that the amount of creatinin in the blood is increased, but he should be able to point out the causes that may be responsible for these conditions, and the bearing that their presence may have on the patient.

But the final word in the diagnosis, treatment, and prognosis of the diseased condition, or conditions, from which the patient suffers rests with the practitioner. It is his function to apply the information furnished by the laboratory to his own interpretation of the condition of the patient, and for this he should not depend upon the laboratory worker. The latter may give him a detailed report of the laboratory findings of the blood, the urine, the

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spinal fluid, or the tissues of the patient, and their variations from the normal, but the formulation of the diagnosis must rest with the clinician. This presupposes a knowledge on the part of the practitioner of the method of interpreting the results of technical procedures.

The purpose of the clinical laboratory is avowedly and properly to aid the physician in his study of the patient, and both physician and laboratory worker should utilize all available means for the advantage of the patient.

Let me indicate the relations that, in my opinion, should exist between the two workers, as well as deprecate some of the present conditions that exist. Their slogan should be *cooperation*. Inasmuch as the aim of both is, or should be, similar, namely, the advantage of the patient, one should help the other to the extent of his ability, and each should act with due consideration for the work of the other, just as two oarsmen should pull their strokes in unison. The problem, therefore, resolves itself into the best method for securing this cooperation. Let me consider, under separate headings, the various factors that go to make up cooperation, it being borne in mind that I speak impartially and not in a spirit of criticism.

1. *When sending material to the laboratory for examination it is well to specify exactly what mode of examination is desired.* The physician's attention is frequently so fully absorbed during the examination of the patient that this simple rule is neglected, and thus much time and labor is lost to the laboratory man. For example, if pus is to be examined for tubercle bacilli, the nature and importance of the examination should be indicated. At times a microscopic examination of a smear for tubercle bacilli is all that is desired, whereas at other times the examination may be of sufficient importance to justify animal inoculation. If, however, this is not indicated at the time the request for examination is made, valuable material that cannot be duplicated may be lost and the laboratory be censured unjustly. Simply to make a request for a bacteriologic examination of a product generally involves unnecessarily a large amount of work. Similarly, the request for "blood examination," so frequently made, is reprehensibly inexact. Under this same heading a word may also be said in regard to leaving laboratory matters to the discretion of internes. I have frequently known a physician, having a leucocyte count in mind, to direct the interne to have a blood examination made, without specifying the type of examination required. Not being quite certain as to what is in the mind of his chief, and desiring to have the information available when called for, the interne directed that all the blood examinations of which he could remember the names at the time be made. True, his chief may thus have been satisfied, but at what unnecessary cost to the laboratory man!

2. *Avoid unnecessary requests.* Examination of the patient should usually take precedence over laboratory investigations. A careful examination of the patient will generally yield information as to what laboratory procedures will best aid in clearing up the case, and in most of the cases only these procedures should be asked for. "Has all laboratory work on the case been done?" This is a question that I have heard repeatedly as the chief was beginning his physical examination. I refer in this place to unnecessary re-

quests because of their inevitable influence on the morale of the laboratory staff. If a laboratory worker has a consciousness that there is a good reason for making every test, his results will, ipso facto, be more reliable and more nearly exact.

3. *The laboratory sciences have their limitations.* This fact should be recognized, and the laboratory should not be held responsible for conditions over which it has no control, or be censured for its inability to do that which is beyond the present scope of the science. This applies perhaps especially to the microscopic examination of tissues. Tissues for examination should be selected carefully, and sufficient information concerning them should be supplied in order to obtain the best results. Frequently if the physician or surgeon is unable personally to visit the laboratory and discuss the case, a brief note on the reverse side of the slip that accompanies the specimen will add a great deal to the information at hand; thus, "This is an interesting case in which there is a question as to diagnosis between a malignant tumor, syphilis, and tuberculosis. The piece is small, it is true, but it is all we could, or will be able to, get." Such a note may lead to animal inoculation for the detection of tubercle bacilli, dark-field examination for treponemata, special fixation for later silver impregnation, in addition to routine morphologic examination. All these would have been impossible if the laboratory man had not thus been forewarned. The absence of such a note has often precluded the possibility of formulating an accurate diagnosis.

4. *The interpretation of results obtained in the laboratory belongs to the province of the physician.* This is true as regards the average case, but, from the nature of his work, in individual or unusual cases the laboratory man is often more skilful in interpreting his results than is the physician, and I have never known a case in which the laboratory worker was not willing to place his knowledge at the disposal of the physician.

5. *Any information that the physician possesses that will aid the laboratory man in making his investigations of a case should be placed at the latter's disposal.* I have known many cases in which such information has intentionally been withheld, at times with the intent of not influencing the laboratory man in making his decision, and at other times because it has been said that, in order to incur favor, the laboratory man would make his diagnosis concur with that of the physician. Of course, such a practice can be regarded only as dishonest. If such a laboratory man exists, he should quickly and forcibly be relieved of his duty. So long as a man is being depended upon to do laboratory work he should be entrusted with all the information that may aid him in his work.

6. *Personal cooperation and consultation between physician and laboratory worker are valuable desiderata.* Personal visits to the laboratory and invitations to the laboratory man to visit the ward and discuss interesting cases cannot fail to be productive of good results to the physician, the laboratory man, and the patient. Real cooperation and an understanding of each other's problems, but not necessarily of one's methods of solution of them, are highly essential. The most successful diagnosticians with whom I

have been acquainted have been men who have made it a practice to visit the laboratory in person with more or less regularity. Whether or not this practice stands in the relation of cause or effect to their success may be a debatable point, but I believe that there is a double relation into which both factors enter.

FROZEN SECTIONS OF TISSUES

Although this topic interests especially the surgeon in his relation to the laboratory, I feel that my presentation of the subject assigned me would be incomplete if this were not referred to. Every laboratory should be equipped for making examinations of frozen specimens of tissue, as the information thus gained cannot be secured in other ways. It is, however, in regard to the advisability and necessity of making frozen sections for diagnostic purposes while an operation is in progress that there is room for a difference of opinion. Personally, it is my belief that facilities for this work should be readily available to every operating room. The cases in which such work should be done as a rapid method of diagnosis for the surgeon is another question. In no phase of laboratory work is cooperation between surgeon and pathologist more essential. In the first place, thorough training in the selection of suitable tissue for examination, and in the interpretation of results secured by this method is a prerequisite on the part of the pathologist. The method is employed most frequently to differentiate tumors and other disease processes, and between benign and malignant tumors, and it is here that this cooperation is most often essential. In differentiating between tumors and other disease processes it is the early cases that give rise to the greatest difficulty, and it is precisely in these cases that numerous foci must be examined carefully before the presence of tumor can be excluded. It is obvious that so careful a search is impossible in the time allowed by a surgeon during operation. In the differentiation between benign and malignant tumors not infrequently the surgeon is in doubt and the pathologist has likewise some difficulty in arriving at a conclusion, since he is restricted in his examination to the small portion of tissue submitted to him. It is on that alone that he can base his opinion. If he finds evidences of malignancy, his verdict is of value. If, however, he finds no evidences of malignancy in the sections he examines, this does not preclude the possibility of malignancy being present. In addition to these groups of cases there are many others in which the pathologist can be of real assistance to the surgeon, and he should ever be ready to lend his aid. My confidence in the discernment of surgeons is great, and I feel that frequently their knowledge of a case is more valuable than any information that the pathologist can furnish them, and their instincts should not be unduly influenced by a doubt raised by a pathologist from the examination of a single specimen, or, at most a few sections hurriedly prepared.

AUTOPSIES

The study of pathologic anatomy has been one of the principal factors in furthering the medical progress that has been achieved since the time of Morgagni, that is, during the latter half of the eighteenth century. It was

Morgagni who first emphasized the importance of comparing the clinical history with the results of autopsy. That autopsies then became frequent is shown by the fact that the classifications of diseased conditions set forth in Rokitansky's "Handbuch der pathologischen Anatomie" in 1846, when he was forty-two years of age, are said to be based on the results of thirty thousand autopsies performed by himself. These figures would tend to show that more than eight autopsies were done every day, including Sundays, during a period of ten years, truly a colossal task. Whether or not these figures are exact, it cannot be denied that this investigator showed an inquisitive spirit of an enviable kind, and set an example that might well be emulated today.

At the present time, in America, the autopsy is not as frequently performed as it should be. For this condition of affairs we as doctors are alone responsible. In Rokitansky's day it was not the layman who demanded the autopsy, but the surgeon himself, who, through his own efforts, obtained the material for autopsies.

Legislation in favor of autopsies may or may not be desirable today, but without specific legislation every physician who attends a dying patient can accomplish more toward securing permission for autopsy than can be achieved in any other way.

The autopsy serves many purposes. Leaving on one side those autopsies that are performed from the medicolegal and sanitary standpoints, let us consider only those that occur in daily practice. More sick and dying are treated in hospitals today than ever before, but the performance of autopsies should not be limited to those dying within the walls of a hospital. Nevertheless, it is to this class of patients that the greatest attention in this respect will be paid for some time to come.

It is my purpose to emphasize the advantages that accrue from an autopsy service, and to state some of the methods by which this service may be increased.

Errors in clinical diagnosis occur daily. Cabot's statistics are probably familiar to you all, and if not, they are easily available. Seeking the causes of these errors, we find that they may arise from several sources, which may be classified as follows:

1. Limitations of medical science.
2. Limitations of our individual knowledge of that science.
3. Inaccuracy of observation.
4. Omission of proper diagnostic procedures.
5. Lapses in mental alertness.

It is obvious that each of these causes of error can be diminished by postmortem investigations, but I will endeavor to emphasize this point by illustrative examples. During a cholera epidemic a man entered the hospital with a weak, fluttering pulse, slight rise in temperature, marked prostration, intense abdominal pain, painful diarrhea, and blood-stained dejecta. The patient was at once transferred to the cholera hospital, where he promptly died. The autopsy disclosed the presence of an acute verrucous endocarditis; a calculus impacted in the cystic duct, and bleeding hemorrhoids, but no

cholera. Regarded from some standpoints, this may have been a justifiable error, but, on the whole, it exemplifies a number of the causes of error. Certainly mental alertness, proper diagnostic procedures, and accuracy of observation, coupled with a knowledge of the diseases that were present, might at least have thrown doubt on the diagnosis of cholera.

It is a truism to be remembered that elimination of causes of error leads to greater accuracy in diagnosis, but let us consider this side of the question for a moment and see to what results it may lead. How would the destructive lesion of the chromaffin system in Addison's disease have been found without the aid of autopsies? How would the condition now termed by some a "subacute bacterial endocarditis" have become a distinct entity without the close correlation of accuracy of clinical observation, mental alertness, and postmortem investigations? And how would we know that our clinical diagnoses were frequently inaccurate if we did not have a Cabot to compare clinical and anatomic diagnoses?

I trust that I have emphasized sufficiently the advantages to be gained from postmortem examinations. That these examinations may be obtained with increasing frequency has been strongly exemplified in our own Philadelphia General Hospital. The methods by which this desideratum has been accomplished have been published and can be studied by all who are interested in the subject. I merely wish here to emphasize that an autopsy is an opportunity, and that that opportunity is yours if you care to grasp it. The pathologist can sometimes be of service in the procuring of autopsies, but the real opportunity lies within reach of him who treats the dying and who comes into intimate contact with the family. A word from the chief can often accomplish much more than the arguments or threats of internes, and the interest that each interne has in securing autopsies, is usually in direct proportion to the interest of his chief.

In conclusion, is it not true that among the elements that contribute to progress in medicine anatomic studies occupy an important place? We are now in the midst of an era of progress. Preventive medicine has made habitable some of the waste places of the earth by attention to epidemiologic factors, such as the elimination of breeding places for vectors of disease, and by the more personal, but also more temporary, expedient of mass inoculations with sera and vaccines. The list of acute infectious diseases that can be controlled by appropriate preventive and curative measures is increasing. X-rays and radium are adding to the effectiveness of treatment and strengthening the arm of the surgeon. Chemotherapy is making possible the successful treatment of diseases which were formerly treated symptomatologically. Organotherapy has attained a place for itself in the list of valuable methods of treatment. Now the question may be raised as to whether or not accuracy of diagnosis is keeping abreast of the general progress. It is true that the laboratory with its newly devised biochemic and serologic reactions is aiding in the recognition of some of the more chronic ailments and the x-rays are helping to diagnose hidden foci of disease. But I have already intimated that the laboratory is a mere accessory in diagnosis, which can best serve those who know best what to search for. This brings us back to the man

who can best interpret symptoms and physical signs, for it is to him primarily that we must look for progress in clinical medicine. It may be profitable for us to inquire of ourselves how much progress we are making along these lines today, and as to whether this progress is commensurate with the progress in other branches. If not, is not the man who habitually correlates his clinical findings and the actual anatomic conditions in a better position to contribute to progress than he who does not? Such correlation is possible through the agency of an efficient autopsy service, and this is an opportunity that each of us can make for ourselves.

THE SEDIMENTATION TEST AS AN AID TO DIAGNOSIS AND PROGNOSIS*

By Herman L. Frosch, B.S., M.D., New York

INTRODUCING a new test to our already overloaded diagnostic equipment, should always be done with some hesitancy, and unless it proves to be of decided value, it will naturally be condemned into the archives of medical history. The sedimentation test, I feel and am convinced, will prove of great assistance to the physician, if interpreted in correlation with his other findings.

It was known as far back as our blood-letting ancestors, that the red cells would agglutinate more rapidly in certain diseases than in others, but it was John Hunter who first wrote about the phenomenon, and since then many researchers have studied it carefully, especially in the past three years by the German and Italian workers.

There are several methods of doing this test. The simplest one, however, is the Linzenmeier technic, which is as follows: Into a 1 c.c. syringe which must be absolutely dry, is drawn 0.2 c.c. sterile sodium citrate, 5 per cent strength. To this is now added 0.8 c.c. of blood drawn directly from a vein. The contents of the syringe are thoroughly mixed and emptied into specially made tubes, 6.5 cm. long, 5 mm. in diameter and a capacity of a little over 1 c.c. Two marks are used, the 1 c.c. mark and another one 18 mm. below it which is called No. III. The time it takes for the red cells to reach mark No. III and leave the plasma above it clear, is called the sedimentation time.

The test was performed on one hundred consecutive cases at Lebanon Hospital. In order to facilitate the studying of the results, the cases will be divided into four groups.

1. Group I will comprise the cases which had a sedimentation time of one-half hour or less.

2. Group II will comprise the cases which had a sedimentation time of between one-half and one hour.

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3. Group III will comprise the cases which had a sedimentation time between one and two hours.

4. Group IV will comprise the cases which had a sedimentation time of more than two hours.

What application can we make from a study of these groups in surgery, in gynecology, in general medicine?

In surgery, any case having a sedimentation time of one-half hour or less should be operated on immediately, because that is an indication that the inflammation is very marked and that waiting spells danger. Just to cite a few cases to prove this contention.

CASE 1.—D. S., twenty-seven years of age, came in with a history of pain in the abdomen, fever, and vomiting of two days' duration. Physical examination revealed marked tenderness and rigidity in the right lower quadrant; temperature 102°; W. B. C., 14,000; P. 85 per cent, L. 15 per cent. The sedimentation time was twenty minutes, at No. III. On operation an acute gangrenous appendix was removed. Eleven days postoperative, the patient had a normal temperature while the cells sedimented down in two hours to No. III.

CASE 12.—M. K., sixty years old. Three months ago, the man fell and struck his right elbow. Subsequently he developed an abscess in that region, which was incised and drained. The pus gave a Streptococcus hemolyticus growth. The infection did not clear up readily and an x-ray showed an osteomyelitis of the olecranon process with a possible suppurative arthritis of the elbow joint. Temperature was 103° F. while the blood sedimented down in ten minutes to No. III. The man was operated on, but died two days later in pulmonary edema following a solid pneumonic process of his entire left lung.

CASE 68.—M. S., thirty-eight years old, came in with a history of having had pain in the abdomen and fever two weeks previously. The pain was most marked in the right lower quadrant. Three days before admission he had a similar attack, vomited several times, following which he had diarrhea. Physical examination showed marked tenderness and rigidity in the right lower quadrant with an indistinct mass in that region. Temperature 102°; W. B. C. 20,000; P. 78 per cent, L. 22 per cent. Sedimentation time ten minutes at No. III. The operation demonstrated an acute appendix with a lateral cecal abscess and it was drained. Seventeen days postoperative it was still draining pus; temperature 101°; sedimentation time forty minutes at No. III. Thirty-six days postoperative, he had a small sinus which was not draining much, temperature 100°, sedimentation time ninety-five minutes at No. III.

In gynecology if we believe that a case of salpingitis should not be opened up during the acute stage, then a sedimentation time of half an hour or less should absolutely not be operated on.

CASE 45.—J. C., twenty years old, came in with a history of abdominal pain vomiting and fever, menses had been irregular and painful. The day before admission she had a sudden chill. Physical examination showed tenderness and rigidity in the right lower quadrant and slight tenderness in left lower quadrant. Vaginal examination showed the cervix pointing anteriorly, uterus enlarged, and retroverted. Motion of uterus was very painful. There was fulness and marked tenderness in right fornix, and slight tenderness in left. The temperature was 101.4° F., hemoglobin 80 per cent, W. B. C. 13,700; P. 82 per cent, L. 18 per cent, sedimentation time fifteen minutes at No. III. Operation revealed a right and left salpingitis, free pus in the right tube. The appendiceal tip was adherent to the right sided inflammatory mass. Microscopic examination demonstrated a normal appendix while the tubes were acutely inflamed. Seventeen days postoperative, the temperature was normal and sedimentation time was two hours and fifteen minutes at No. III.

CASE 64.—P. L., twenty-two years old came in with tenderness and rigidity in left lower quadrant, fulness in left fornix on examination. Temperature 99°; hemoglobin 70

per cent; W. B. C. 12,000; P. 80 per cent, L. 20 per cent, sedimentation time forty-five minutes at No. III. Operation showed a left salpingitis well walled off from surrounding viscera. The pathologic diagnosis was acute inflammation. Sixteen days postoperative, the temperature was normal and sedimentation time was two hours and thirty minutes at No. III.

No case of adnexal disease should be operated on, unless sedimentation time indicates that all latent foci of infection have been eliminated. Especially should the sedimentation test be used in those cases of adnexal disease which have a normal temperature and normal blood count. A rapid sedimentation time means infection and the operation should be delayed until the test indicates a sterile field.

Can the test be used in general medicine? A study of Tables I, II, III and IV will demonstrate conclusively its importance in medical cases; first, as an aid in prognosis, second, as to the progress of the case. The prognosis should be very guarded in a case having a sedimentation time of less than half an hour. The test done at different intervals of the illness will show whether the patient is improving, or is getting worse. Just to cite a few cases to demonstrate this contention.

CASE 2.—M. S., came in with the following history: One week previous to admission he developed precordial pain, sweats and chills and temperature 102° to 103°. He had had frequent sore throats previous to that for which tonsillectomy was performed two months before. Physical examination showed the following: conjunctivitis, right pupil greater than left, systolic and diastolic murmurs at base of heart. He developed transient pains in left knee and right wrist, but no redness or swelling was present. A culture of the blood gave a Streptococcus viridans growth with 500 colonies per 1 c.c. of blood. His temperature was 103° and sedimentation time one hour at No. III. Subsequently he developed a phlebitis of the right antecubital region, bright red blood in his stools, petechial hemorrhages over his lower dorsal region, blood in the urine, splenic infarct and a perisplenitis. His sedimentation time was forty-five minutes. One month later he had a cerebral embolus with a paralysis of the left side of the body and face. Sedimentation time following that was twenty-five minutes at No. III. Four days later the sedimentation time was ten minutes at No. III. He expired four hours later.

CASE 3.—M. M., came in with a history of fever, dyspnea and cough four weeks before admission following exposure to cold and rain. He was subject to sore throats three or four times a year. Seven years ago he had an attack of rheumatic fever. Physical examination showed enlarged, red tonsils, systolic and diastolic murmurs heard all over precordium, accentuated P2, pulse deficit 16, and sibilant râles in his lungs. He was fibrillating, temperature 102°; W. B. C. 14,000; P. 83 per cent, L. 17 per cent, sedimentation time was one-half hour at No. III. Three weeks later the patient was clinically very much better. His temperature 99.6° F., sedimentation time one hour at No. III. Blood culture was sterile. Four weeks later the temperature was normal and sedimentation time one and one-half hours at No. III.

Briefly, an increasing sedimentation time in a patient means improvement. A diminishing sedimentation time means that your patient is getting worse, even though the clinical picture indicates improvement.

The temperature does not in any way affect the sedimentation time. In one of the typhoid cases with a temperature of 104° to 105° F. the sedimentation time was one and one-half hours at No. III, while in a patient with an acute appendicitis whose temperature was 100° F. the sedimentation time was twenty minutes at No. III.

GROUP I

CASE	DIAGNOSIS AND CLINICAL CONDITION	SEDIMENTATION AT DIFFERENT INTERVALS IN MINUTES TO NO. 3 AND COMMENT
1	Acute gangrenous appendicitis	20 min. 11 days P.O.*—120 min.
77	Secondary appendiceal abscess	20 " Before discharged, 90 min.
11	Acute appendicitis with general peritonitis	15 " 7 days P. O.—105 min.
13	Acute appendicitis	15 " 11 days P. O.— 90 min.
33	Acute appendicitis with general peritonitis	15 " 1 day P. O. 30 min. 18 days P. O. 150 min.
41	Acute gangrenous appendicitis	25 " 1 day P. O. 60 min. 13 days P. O. 135 min.
53	Acute appendicitis	2 days P. O. 30 min. 10 days P. O. 65 min.
68	Acute appendicitis with lateral cecal abscess	10 " 17 days P. O. 40 min. 36 days P. O. 95 min. Drain in small sinus
82	Acute appendicitis	105 " Patient not operated
86	Acute gangrenous appendicitis with local peritonitis	20 " 1 day P. O. 35 min. 10 days P. O. 70 min.
96	Acute appendicitis	13 " 1 day P. O. 52 min. 13 days P. O. 105 min.
3	Acute rheumatic endocarditis fibrillating, left pulmonary infarct, sore throat. Blood culture sterile	30 " 3 weeks later 60 min. 4 weeks later 90 min. Patient discharged
8	Perirectal abscess	12 " 7 days P. O. 120 min.
10	Chronic myocarditis, auricular fibrillation, embolism left femoral	Amputation of left leg, right sided complete hemiplegic attack, 30 min. 3 weeks later 120 min.
12	Suppurative arthritis and osteomyelitis	10 " Patient died
18	Thromboangiitis obliterans, infection right leg, amputation of right leg	13 " 9 days P. O. 10 min. 31 days P. O. 125 min. Stump infected
20	Double aortic and mitral regurgitation; acute rheumatic fever;	26 " 1 day before death 10 min.
23	Internal hemorrhagic pachymeningitis	30 " Before discharge 150 min.
28	Cardiac insufficiency, chronic myocarditis, subacute rheumatic fever, pneumonia	10 " 10 days later 12 min.
29	Carcinoma of stomach	18 "
31	Subaponeurotic abscess of scalp	15 " 11 days P. O. 45 min. 14 days P. O. 90 min.
36	Acute articular rheumatism	14 "
38	Persistent obstipation and vomiting; severe cold	15 "
40	Pelvirectal abscess; pulmonary tuberculosis	22 "
45	Bilateral pyosalpinx	15 " 17 days P. O. 170 min.
46	Lobar pneumonia, type IV	13 " 10 days later 190 min.
47	Acute rheumatic fever	15 " 3 weeks later 105 min.
48	Abscess of lung	15 " 5 weeks later 45 min.
50	Cardiac insufficiency; chronic myocarditis; bronchopneumonia	22 " Expired
56	Cellulitis of forearm	30 " 4 days later 135 min.
58	Chronic cholecystitis; cirrhosis of liver	15 "
59	Complete general arteriosclerosis	25 " Later 45 min.
60	Diabetes; cardiac hypertrophy; chronic bronchitis and myocarditis decompensated	25 "
78	Cellulitis	15 " 2 days P. O. 25 min. 8 days P. O. 65 min.
81	Common duct obstruction and jaundice	28 "
89	Nephrolithiasis with pus	25 "
93	Pyonephrosis	18 " 2 days before death 10 min.
95	Lobar pneumonia	11 " 1 week before discharge 180 min.
64	Left salpingo-oophoritis	22 " 7 days P. O. 45 min. 16 days P. O. 135 min.

*P.O.=postoperative.

Is the sedimentation test any improvement on our usual blood count? I think so. One case will demonstrate how the test proved to be right.

CASE 11.—J. B., came to the hospital with a history of vomiting, abdominal pain and fever of two days' duration. Physical examination showed tenderness and rigidity in the lower half of the abdomen. Operation revealed an acute appendicitis with a general peritonitis. Four days postoperative his temperature came down to normal and stayed there until eleven days after operation when it went up to 102° F. Examination revealed tenderness in right flank. A blood count showed W. B. C. 10,000; P. 67 per cent, L. thirty-three per cent. Certainly his blood count does not indicate an inflammatory condition. But a sedimentation test taken the same day showed that the red cells came down to No. III in fifteen minutes. On opening the abdomen an abscess was found situated between the parietal peritoneum, the cecum and ascending colon containing thick greenish pus. The temperature came down promptly. One week later the sedimentation time was two and three-fourths hours at No. III.

GROUP II

CASE	DIAGNOSIS AND CLINICAL CONDITION	SEDIMENTATION AT DIFFERENT INTERVALS IN MINUTES TO NO. 3 AND COMMENT
2	Acute endocarditis, aortic stenosis and regurgitation, blood culture Streptococcus viridans	3 weeks later splenic infarct and perisplenitis 45 min. 60 min. 2 months later cerebral embolus 25 min.; died
4	Pyelitis	35 " 4 days later 25 min., 7 days later 90 min.
6	Chronic cholecystitis and chronic appendicitis	40 "
14	General intraabdominal adhesions and chronic bronchitis	40 "
15	Suppurative axillary adenitis	55 " 3 days P. O.* 105 min.
27	Typhoid fever	53 "
42	Medial ventral hernia	50 " 12 days P. O. 175 min.
76	Acute gastroenteritis	55 "
85	Incomplete abortion	45 "
63	Chronic appendicitis	60 " 11 days P. O. 115 min.
84	Intramammary abscess	55 " 2 days P. O. 115 min.

*P.O.=postoperative.

GROUP III

CASE	DIAGNOSIS AND CLINICAL CONDITION	SEDIMENTATION AT DIFFERENT INTERVALS IN MINUTES TO NO. 3 AND COMMENT
9	Right indirect inguinal hernia, and hydrocele of canal of Nuck	2 days P. O.* 60 min. 85 min. 12 days P. O. 150 min.
37	Typhoid fever	90 "
43	Calculus in ureter	115 "
44	Neurosis of intestines	120 "
52	Cellulitis of face	90 "
54	Fracture of right tibia	80 "
57	Acute arthritis of left hip	80 "
61	Ruptured duodenal ulcer	1 day P. O. 90 min.
65	Salivary calculus; and acute suppuration, sublingual adenitis	105 "
70	Laceration and crushing of fingers	110 "
75	Syphilis of liver, hemiplegia	105 "
79	Contusion of right leg	93 "
80	Undetermined diagnosis	105 "
83	Fracture of tibia and fibula	85 "
91	Chronic appendicitis	100 "
92	Fracture of rib	120 "
94	Resolving lobar pneumonia	65 "
73	Acute suppurative adenitis	75 " 4 days P. O. 135 min.
99	Typhus fever(?)	90 " Four days later 135 min.

*P.O.=postoperative.

GROUP IV

CASE	DIAGNOSIS AND CLINICAL CONDITION	SEDIMENTATION AT DIFFERENT INTERVALS IN MINUTES TO NO. 3 AND COMMENT
5	Lipoma of neck	240 min.
16	Hemorrhoids	190 "
17	Compound fracture of skull	125 "
19	Mitral stenosis	150 "
22	Partial heart block	187 "
24	Acute nephritis and myositis	180 "
30	Gastrojejunal ulcer	125 "
32	Left oblique inguinal hernia	125 "
34	Fistula in ano	360 "
35	Contusion of left side of face	220 "
39	Hemorrhoids	390 "
49	Hemorrhoids	240 "
51	Influenza	Five days before discharge 300 min.
55	Neurosis of heart	300 "
62	Neurosis of stomach	405 "
66	Probably chronic appendicitis	150 "
67	Fracture of skull	135 "
69	Chronic appendicitis(?)	480 "
71	Hemorrhoids	180 "
74	Fracture of tibia and fibula	195 "
77	Hemorrhoids	540 "
87	Nephrolithiasis	135 "
88	Indirect inguinal hernia	150 "
90	Cystic hypertrophy of endometrium	135 "
97	Chronic interstitial nephritis	210 "
98	Chronic myositis, tonsillitis, and pharyngitis	480 "

In order to determine which portion of the blood causes the change in the sedimentation time, that is, whether the cause is in the plasma or in the red blood cells, the following experiments were performed:

The serum of a patient whose sedimentation time was ten minutes was intimately mixed with the red cells of a patient whose sedimentation time was two and one-half hours in normal proportions. This mixture sedimented down to No. III in twenty minutes. The reverse experiment was also performed by adding the serum of the latter case to the cells of the former. In this case the sedimentation time was two hours and ten minutes at No. III. This type of experiment was repeatedly performed and gave the same results. The only conclusion that can be derived from the above, is that the rate of sedimentation is dependent upon some constituent in the plasma rather than in the red cells.

It is a well-known fact that in certain diseases as pneumonia, acute articular rheumatism, the various suppurative conditions, and in diseases where tissue destruction is occurring there appears in the plasma of the blood a relative increase in globulin and fibrinogen, with a relative diminution in albumin. This change results in a diminution of surface tension, which also means a diminution in the cohesive powers between the individual molecules making up the plasma, and thus the red cells come down faster than in a normal individual.

There are at present three outstanding theories as to the cause of the quickening of sedimentation in certain diseases.

Fahrenaus and Huber regard it as due to a change in the electric charge of the red cells. Normally they carry a negative charge and thus they repel

each other. In certain diseases this negative electric charge is diminished. This diminishes the repelling qualities for each other, facilitating agglutination and sedimentation.

Plaut seeks an explanation of this phenomenon in increased autoagglutination which he thinks is due to an increased fibrinogen content of the plasma.

Sachs claims that it is due to a variation in the stability of the colloids of the plasma in which globulin and fibrinogen are the most changeable components.

Wilhelm Starlinger performed a very clever and ingenious experiment a few years ago. He added kaolin, which absorbs fibrinogen to a blood whose sedimentation time was very rapid. He then showed that the sedimentation time of that blood was very much increased after such procedure.

After a thorough study of the literature as to the behavior of colloids, it seems to me that all three theories are correct. Primarily, there is a relative increase of globulin and fibrinogen in certain diseases. These particular colloids are composed of larger molecules than the other usual colloids of the blood. Thus, there is a diminution in the cohesive power between the molecules making up the plasma. Furthermore, these colloids also have a greater absorption power for the alkaline salts in the plasma and thus the negative electric charge of the red blood cells is diminished, which is conducive to more rapid agglutination of the cellular elements of the blood. Briefly, an increase in globulin and fibrinogen produces a lower surface tension of the plasma and a more rapid agglutination of the red cells, and thus a more rapid sedimentation time in those conditions in which these two colloids are increased in the plasma.

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A COMPARATIVE STUDY OF THE KOLMER COMPLEMENT-FIXATION TEST FOR SYPHILIS WITH SERA BEFORE AND AFTER THE REMOVAL OF ANTISHEEP HEMOLYSIN*

By Susumu Uchida, M.D., Philadelphia, Pa.

FOR many years, there has been diverse opinions as to the value of absorbing the natural antisheep hemolysin from sera before conducting the complement-fixation test for syphilis. That an appreciable amount of antisheep hemolysin is present in human sera has been demonstrated by numerous investigators. A detailed résumé has been compiled from the available literature on this subject by Sachs.[1] Kolmer and Casselman[2] have titrated the thermostabile hemolysins for corpuscles of various vertebrates in a large number of specimens of human sera. They observed that in over 80 per cent of these sera, there is present sufficient natural antisheep hemolysin to give well marked or complete hemolysis. Van Saun[3] obtained similar results in a large proportion of cases (51 per cent). Working with a series of over 6,000 samples of sera which had been submitted for routine complement-fixation tests for syphilis, only 12 per cent were found by Gilbert and Wemple[4] to contain appreciable amounts of natural amboceptor (1924). Our own results are more nearly in accord with those of the former, our percentage being approximately 60 per cent.

Bauer[5] and Hecht-Weinberg[6] therefore advised the modification of the Wassermann reaction on those samples of sera already containing the proper dose of hemolytic amboceptor, whereby this natural amboceptor is utilized, with no need for the addition of an immune amboceptor. In this modification, none of the syphilitic antibody is destroyed and no complementoid produced. Kolmer proved that the Hecht-Weinberg modification is quite delicate because of this, but claims that false complement fixations occur when crude alcoholic extracts of the organs are used as antigens. To obviate this Noguchi[7] made use of an antihuman in place of the antisheep hemolytic system; but he overlooked the possibility of a natural antihuman hemolysin, which Williams[8] has shown to be present in some sera.

The question therefore arises: Shall or shall we not absorb this natural antisheep hemolysin before adding the artificial hemolytic system? Is the sensitiveness of the test affected by its presence or absence? This we have endeavored to answer by conducting a series of experiments whereby the complement-fixation test was performed on human sera prior to and after the absorption of the natural antisheep hemolysin. We also wished to confirm or deny the results of other workers who had not used Kolmer's new

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method for complement fixation with and without this absorption. While some investigators have found that the effect of the natural amboceptor is so slight that it can be considered negligible, there are others who quite disagree with this conclusion. Much depends upon the factors of technic employed, such as the test of serum complement and amboceptor, the duration of the primary incubation period and the time at which the final reading is made, whether at once or after standing several hours or overnight.

Kolmer and Rule[9] have studied in detail the factors affecting the ultimate readings of the test. Strongly positive sera, containing large amounts of syphilitic reagin are not influenced in quantities of 0.1 and 0.2 c.c., by the presence of the antisheep hemolysin naturally contained in the sera. However, sera with relatively small amounts of syphilitic antibody and large amounts of natural amboceptor may yield falsely negative reactions, unless the natural hemolysins are removed. The effect upon weakly positive sera is not as pronounced when the readings are made immediately after the second period of incubation, as when they are made after the tubes had been placed in a refrigerator for sixteen hours or longer.

A recent paper has appeared by Gilbert and Wemple[4] in which are reported similar results to those given by Olmstead[10] and Kolmer and Rule.[9] They are agreed that the influence of natural amboceptor is less apparent if the complement-fixation tests are read immediately after the second incubation than if read the next morning. But Olmstead[10] concluded from the researches that if the tubes are read as soon as the serum and antigen controls are completely hemolyzed, the natural amboceptor content of the serum makes but little difference in the Wassermann test. The removal of natural hemolysin, in his opinion, does not appear to be essential to the sensitiveness of the test.

Those[7, 23, 24] who employ an antihuman hemolytic system appear convinced that it is more sensitive owing to the absence of these natural antisheep hemolysins, in contradistinction to those who, using the antisheep hemolytic system, deny or minimize the influence of the natural amboceptor in the technic to which they adhere. The addition of artificial antisheep hemolysin is unnecessary, owing to the natural amboceptor present, for the production of hemolysis in the serum control tubes. Theoretically, at least, the presence of unknown natural amboceptor in some sera, will obscure evidences of its quantitative relations to the antisheep hemolytic system, and the reactions may still be inaccurate. There are those, therefore, who deem it a more satisfactory solution of the problem to remove the natural hemolysin from the sera before the performance of the test, so that the balance of the hemolytic system may be assured by the addition of the known quantity of amboceptor.

It must be remembered that the authors cited above used the Wassermann complement-fixation technic. Only one investigator (Kilduffe[11]) applied the absorption of the natural amboceptor to Kolmer's new method so widely advocated. This technic is characterized by a very high degree of specific sensitiveness, by almost no tendency to yield apparently nonspecific reactions, and by a very low percentage of anticomplementary reactions

(Schamberg and Klauder,[12] Shivers,[13] Kilduffe,[14] Palmer and Gibbs,[15] Smith[16] and others).

It might not be amiss to give in detail Kilduffe's[11] conclusions. I may add that our own experiments were in progress when these results were published. It was, therefore, of great interest to us to learn whether or not our findings tallied with his. He states, "The presence of large amounts of natural antisheep hemolysin is not without effect upon the reaction in Kolmer's quantitative complement-fixation test for syphilis, even when the serum contains relatively large amounts of syphilitic reagin, and may give rise to false negative results in approximately 10 per cent of hemolysin containing sera." He thus concurs with the opinion of those who had used the old method, and advises that the natural antisheep hemolysin be absorbed from the serum prior to the performance of Kolmer's quantitative complement-fixation test, and so increase the delicacy of the reaction.

EXPERIMENTAL

In all probability, the complement-fixation test for syphilis serves its most useful purpose in the diagnosis of the disease in its clinically latent and tertiary stages, as a guide in treatment and a criterion of cure. The very nature of the pathologic and immunologic changes in chronic and latent syphilis demands that the complement-fixation test shall be consistent with specificity to render its best service, as an aid in diagnosis. Therefore, it is essential to determine the exact part the antisheep amboceptor plays in the sensitiveness of the test. Before ascertaining this, we thought it necessary to confirm the findings of other workers as to the percentage of human sera found to contain antisheep hemolysin (0.1 c.c. human serum was used throughout in the serum control). We had at our disposal, numerous patients from the out-door department of the Polyclinic Hospital, of Philadelphia, which included specific and nonspecific cases, the former comprising all stages of the disease from the frank secondaries and primary to the tertiary and latent syphilis. Many methods for the absorption of antisheep hemolysin are in vogue. Among the earlier workers were Bauer,[17] Rossi[18] and Jacobaeus.[19] They advocated the use of homologous corpuscles, but this procedure, according to Bauer,[17] frequently rendered the serum anticomplementary. This is known as the Sachs-Friedberger phenomenon, and is counteracted by the absorption of heated sera at a low temperature (Rossi[18]). Rossi, therefore, makes use of the refrigerator in the active part of the absorption. All investigators in this phase of the work utilize sheep's cells, but each in turn had so modified the original procedures as to render them rather time consuming and laborious. Among the latter is Simon[20] who advocates the use of varying amounts of serum in his test. This method has been used by Kolmer, who found it very satisfactory, showing but an occasional anticomplementary result. This latter unfavorable condition has been combated by Kahn[21] in his excellent method of allowing the absorption process to take place in but ten minutes at room temperature, followed by centrifuging and storing in the refrigerator for sixteen to eighteen hours. This technic has been highly recommended by Kilduffe.[11] Gilbert and Wemple[4] described a

similar nontime-consuming procedure which they claim produces no anti-complementary properties; they make use of the refrigerator as does Rossi (3° 6° C.).

The ideal method for removing antisheep amboceptor from human sera should be efficacious, rapid and simple, and with the added attribute of not rendering the sera anticomplementary. We have attempted to devise a method which would come closest to the required properties. Our technic was as follows: A series of 500 sera was tested for the presence of antisheep hemolysin. The serum of each specimen to be examined was divided into 2 equal parts. One portion was tested "untreated" but inactivated at 55° C. for fifteen minutes. The other was subjected to the following: To 1 c.c. of serum we added 2 drops of washed well-packed sheeps' cells (from a 1 c.c. pipette); the mixture was allowed to remain at room temperature for fifteen minutes. After centrifuging to separate the "treated" serum from the cells, this serum was inactivated at 55° C. for fifteen minutes. It now remained to test whether the natural antisheep amboceptor had been absorbed. To 0.1 c.c. of the "treated" serum was added 1 c.c. of 2 full units of complement and sufficient isotonic salt solution to make 2 c.c. The next day, after the mixture had remained in the refrigerator for sixteen to eighteen hours, 0.5 c.c. of a 2 per cent suspension of sheep cells was added and incubated for one hour at 38° C. (It will be noted that no artificial hemolysin was used.) Reading of the tube was made immediately after incubation. There was complete inhibition of hemolysis showing that the hemolysin for the corpuscles employed was absent. As a control, the untreated inactivated serum was subjected to the same procedure, with resulting complete hemolysis in the majority of the cases, i.e., in those sera which contained a sufficient amount of natural antisheep amboceptor. This absorption method satisfied all requirements and was used throughout the experiment.

Before the above technic was finally decided upon, we experimented with varying periods of sensitization of sheep cells and active serum; we obtained frequent unsatisfactory results in those cases in which sensitization took place for more than twenty minutes, owing to discoloration of some sera. Good results were obtained with contact at fifteen minutes, which procedure we adhered to.

To return to our comparative study of the complement-fixation reaction of the new Kolmer technic for syphilis, with sera before and after the removal of antisheep hemolysin. We now subjected the "treated" and "untreated" sera simultaneously to the standardized Kolmer method, using the cholesterinized lecithinized antigen with 1 c.c. of two full units of complement and with the proposed primary incubation period of sixteen to eighteen hours at 6° to 8° C., followed by five to ten minutes in the water bath at 38° C. The hemolytic system comprising 0.5 c.c. or two units of hemolysin and 0.5 c.c. of 2 per cent suspension of sheep cells was added prior to the second incubation period of one hour at 38° C. The tubes were read immediately thereon.

RESULTS

In a series of 500 sera, 215 or 43 per cent produced complete hemolysis; 84 or 16.4 per cent showed very marked hemolysis. We concluded that about 60 per cent of the sera had contained sufficient natural antisheep hemolysin. Of the series, 77 or 15 per cent possessed no hemolysin at all.

Table I gives the summary of the results observed in the Kolmer complement-fixation tests before and after absorption of the natural antisheep hemolysin. It concerns the percentages calculated on the total number of sera examined (500), 75.8 per cent exhibiting no change in the reading. These include negative sera. Eight per cent manifested positive reactions with treated sera, when the untreated sera had given negative results. Relatively stronger reactions (in degree) were seen in 7 per cent, while only 1.2 per cent showed weaker reactions after the natural antisheep hemolysin had been absorbed.

TABLE I

RESULTS OBTAINED IN KOLMER COMPLEMENT-FIXATION TEST IN 500 SERA BEFORE AND AFTER ABSORPTION OF NATURAL ANTISHEEP HEMOLYSIN

	SERA SHOWING NO CHANGE AFTER ABSORPTION	POSITIVE REACTION AFTER ABSORPTION	STRONGER REACTION AFTER ABSORPTION	DOUBTFUL REACTION AFTER ABSORPTION	WEAKER REACTION AFTER ABSORPTION	ANTICOMPLEMENTARY ACTIVITY AFTER ABSORPTION
PERCENTAGE	75.8	8	7	0.4	1.2	6.2
NUMBER	379	40	35	2	6	31

TABLE II

RESULTS OBTAINED IN KOLMER COMPLEMENT-FIXATION TEST AFTER ABSORPTION OF NATURAL ANTISHEEP HEMOLYSIN

NUMBER OF REACTING SERA AFTER ABSORPTION	NUMBER OF SERA SHOWING NO CHANGE IN DEGREE OF FIXATION	SERA SHOWING INCREASED DEGREE OF FIXATION AFTER ABSORPTION			NUMBER SHOWING WEAKER REACTIONS AFTER ABSORPTION
		TOTAL NUMBER OF SERA	NUMBER OF POSITIVE AFTER ABSORPTION. (NEGATIVE BEFORE)	NUMBER SHOWING STRONGER REACTIONS AFTER ABSORPTION	
263	142	74	40	34	6

The total number of sera specimens showing anticomplementary properties after absorption was 38; seven of these possessed these properties before the removal of the hemolysin. Therefore, 6.2 per cent acquired anticomplementary changes during the procedure of "treating" the sera. Doubtful reactions in which the first tube was ±, with the serum control giving complete hemolysis, were obtained in 0.4 per cent of the cases.

There were 263 samples of sera which reacted positively both before and after absorption; of these, 142 or 53.9 per cent showed no change in the degree of fixation of the complement by the syphilitic reagin before and after treatment of the sera. An increase in the amount of fixation of the complement manifested itself in a total of 74 samples of sera, 40 of which had been negative before removal of the hemolysin. The remaining 34 re-

vealed stronger positive reactions after absorption than they had shown before. However, in 6 of the sera specimens, a weaker reaction had occurred.

The 40 specimens of sera which gave positive reactions after absorption with previous negative ones before absorption, showed varying degrees of inhibitive hemolysis. Twenty-three of these reacted positively in the first tube and seven in the first three tubes. None of them reacted in the fourth or fifth tubes (Table III).

TABLE III

SERA SHOWING WEAKLY POSITIVE REACTIONS AFTER ABSORPTION

1 - - - -	1 - - - -	3 - - - -	2 - - - -
1 - - - -	1 - - - -	1 - - - -	2 - - - -
2 - - - -	4 - - - -	2 - - - -	2 - - - -
1 - - - -	2 - - - -	1 - - - -	1 - - - -
1 - - - -	1 - - - -	1 - - - -	1 - - - -
2 - - - -	1 - - - -	2 - - - -	

SERA SHOWING MODERATELY POSITIVE REACTIONS AFTER ABSORPTION

2 1 - - -	1 1 - - -	4 3 - - -	3 2 - - -	3 1 - - -
2 ± - - -	3 ± - - -	2 1 - - -	2 1 - - -	2 1 - - -

SERA SHOWING STRONGLY POSITIVE REACTIONS AFTER ABSORPTION

2 1 1 - -	4 4 2 - -	4 4 2 - -	2 1 1 - -
4 4 3 - -	3 2 1 - -	3 2 1 - -	

1=+ 2=++. 3=+++. 4=++++. -=negative; ± = doubtful.

TABLE IV

THE RELATION BETWEEN THE CLINICAL DIAGNOSIS AND POSITIVE SERA AFTER ABSORPTION

CLINICAL DIAGNOSIS	NUMBER OF TUBES SHOWING REACTION AFTER ABSORPTION			
	Positive 1 Tube	Positive 2 Tubes	Positive 3 Tubes	Total
No history	2	0	1	3
No syphilis	4	1	0	5
Primary syphilis	2	0	0	2
Secondary syphilis	2	2	0	4
Latent syphilis	9	6	6	21
Congenital syphilis	1	1	0	2
Suspicious	3	0	0	3

Positive 1st tube=weakly positive. Positive 2 tubes=moderately positive. Positive 3 tubes=strongly positive.

TABLE V

COMPLEMENT-FIXATION RESULTS BEFORE AND AFTER ABSORPTION

CASE NO.	CLINICAL DIAGNOSIS	DATE	KOLMER COMPLEMENT FIXATION	
			Before Absorption	After Absorption
3	Latent Syphilis	10- 5-23	- - - - -	2 1 1 - -
		10-12-23	- - - - -	1 1 - - -
		11-14-23	- - - - -	2 1 1 - -
5	Latent Syphilis	10-10-23	- - - - -	2 1 - - -
		10-26-23	- - - - -	3 2 - - -
7	Latent Syphilis	10-12-23	- - - - -	3 - - - -
		10-26-23	- - - - -	3 1 - - -
10	Non-Syphilis	10-24-23	- - - - -	1 - - - -
		11-14-23	- - - - -	2 1 - - -
15	Latent Syphilis	10-26-23	- - - - -	4 4 2 - -
		11-27-23	- - - - -	4 4 3 - -
20	Secondary Syphilis	11- 2-23	- - - - -	2 - - - -
		11- 9-23	- - - - -	3 2 - - -

It was interesting to investigate the clinical data of the cases from which the sera with negative complement-fixation results had become positive after the natural amboceptor had been removed. Table IV gives the relation between the clinical diagnosis and the corresponding sera yielding positive sera after absorption had taken place, but negative sera before. There were 40 cases examined, which embraced nonsyphilitics, suspicious cases as well as patients suffering from all stages of lues. Twenty-one of the total number of 40 were latent syphilitics. In all of these, the positive reaction was elicited after "treatment" of the sera in contrast to the negative results prior to this removal of the amboceptor. Tests were conducted on sera withdrawn on separate occasions from the same patients later found to be suffering from various stages of lues. Protocols are given in Table V and show that these positive reactions after absorption were not sporadic, but remained consistent in the various tests made at periodic intervals. All these luetic patients, most of whom were latent syphilitics, were receiving treatment during the conduction of these experiments.

DISCUSSION

From our experiments, we deducted the fact that of all the fresh inactivated human sera examined, a large proportion contained sufficient natural hemolysin to completely or markedly hemolyze the usual dose of sheep cell suspension with its customary amount of guinea pig complement. There is a difference of opinion, however, as to the exact percentage of cases containing natural hemolysin, some workers claiming that there is only a small and insignificant number of such sera. This diversity of opinion is probably due to the different technics followed, varying amounts of complement and sheep cells used, and whether short or long incubation periods had been advocated. It will be readily understood that the presence of an unknown amount of natural antisheep hemolysin in sera would be a drawback to accurate quantitative estimations. Even an excess of 4 or 5 units of antisheep hemolysin will so influence the reactions as to cause an occasional false reaction to occur or a positive result to be lost (Noguchi,[7] Olmstead,[10] Bailey[25] and Kolmer[9]).

It is the opinion of Kolmer and Rule[9] that an appreciable amount of natural hemolysin in the presence of large quantities of syphilitic reagin will be insufficient to mask the detection of complement-fixing bodies. These findings have been confirmed by Kilduffe.[11]

The results of our own experiments (Table III) show that out of the 40 samples of sera which gave positive results after the removal of the hemolysin and negative before, 23 were weakly positive in the first tube. We therefore concluded that small amounts of syphilitic reagin are masked by the large amount of antisheep hemolysin naturally found in sera. The remaining 17 tubes showed a moderately positive reaction. The latter thus contained relatively larger amounts of syphilitic reagin and were also influenced by the natural amboceptor. The results of the complement-fixation tests (Kolmer's new method) on all the 40 sera specimens, before "treatment," were negative, suggesting the conclusion that whether small or large

amounts of syphilitic reagin are contained in the sera, the presence of natural antisheep hemolysin will affect the readings.

This mechanism is due to one of two explanations. It may lie in the fact that for some unknown reason an excess of hemolysin may completely hemolyze the corpuscles, even though a small amount of the necessary complement has been specially fixed by the antigen and the syphilitic antibodies, or it may be due to the fact that large amounts of hemolysin prevent some of the complement from joining with the antigen and syphilitic antibodies, and this renders the complement free. It is the opinion of a number of research workers that absorption of the sera does not remove the syphilitic reagin.

Two hundred and sixty-three of our series of 500 revealed positive reactions before removal of the antisheep amboceptor. Six of these showed weaker positive results after treating the sera with sheep corpuscles, but falsely negative readings after absorption were never obtained throughout the experiment.

These weaker positives are probably due to other factors rather than heating to 55° C. It is not avoidable to eliminate errors attributable to slight inaccuracies from pipetting the exact quantities indicated. Such diminutive amounts of packed sheep cells are used (2 drops) that any salt solution added in the washing of the corpuscles that had not been removed, may so dilute the serum as to mask the reaction. Care should be taken to rule out this possibility and to discard as much of the saline as is possible. In spite of this caution Van Saun[3] claims that the antibody content of the serum in a few instances is weakened by the absorption treatment.

It is generally admitted that there may be a development of anticomplementary properties in an occasional serum after absorption with sheep corpuscles. Some little difficulty has been encountered in finding a method satisfactory for the complete absorption of hemolysin without the coincident development of the thermostabile anticomplementary properties in the serum after absorption. Sach's explanation of the Sachs-Friedberger phenomenon is that the serum possesses a so-called antagonistic nature in addition to the natural hemolysin. This nature is produced by the "Hemmende anti-Korper" and causes the anticomplementary activity, being enveloped by the normal amboceptor.

According to Rossi, methods advocating the use of incubator temperature removes only the normal amboceptor. Low temperatures for sensitization do away with both the natural hemolysin and this antagonistic nature of the serum. His recommendation of low temperature rests on this factor. Kahn advocates ten minutes at room temperature followed by storing in the ice chest overnight and claims to have eliminated anticomplementary results with this procedure. We have usually removed anticomplementary substances by heating the "treated" sera to 55° C. for fifteen minutes immediately prior to the complement-fixation test. We have been able to demonstrate that only 31 out of the 500 sera specimens of our series or 6 per cent contained thermostabile antilysin which was not influenced by fifteen minutes exposure to 55° C. From the viewpoint of Rossi it may be that low

temperature for the sensitization of the sera may be advisable, in spite of the extra time involved.

Two methods have been advocated for the absorption of the natural antisheep amboceptor by the sensitization with sheep cells, the main differing factor being the use of active or inactivated sera. It is conceded that, by subjecting the sera to the fifteen minute exposure to 55° C. for inactivation, some of the thermolabile reacting bodies contained in the sera are destroyed. The second heating to 55° C. used in the process of absorption further removes those bodies which had escaped the first heating. The total exposure to 55° is not more than thirty minutes, no longer than that formerly in use in the Wassermann technic. We have employed only active sera in our sensitization process with sheep cells and have allowed extraction to take place at room temperature instead of the refrigerator; for it is our belief that the one heating to 55° for fifteen minutes during the period of absorption is sufficient to remove the native natural complement in human sera, with no production of complementoid. The thermolabile reacting bodies are thus little destroyed. The sensitization of the sheep's corpuscles is more rapid when taking place at higher temperatures than at lower. The length of time allowed for this is important, owing to the likelihood of discoloration occurring in the sera when there is too long an elapse of time. After experimenting with different time intervals, we found that the fifteen minutes at room temperature is best for our purpose, thirty minutes causing a tinge of discolor in the sera.

Although we must admit that we have obtained an occasional anticomplementary reaction, there is no doubt that our technic of the removal of natural antisheep hemolysin does increase the sensitiveness, and enhance the delicacy of the complement-fixation test. With further concentration to perfect the method this disadvantage may be counteracted.

It has been observed that the Kolmer new standardized technic will yield an occasional anomalous reaction. In a definite proportion of these anomalously reacting sera, the reaction is due, according to Kolmer, partly to the presence of relatively large amounts of natural antisheep hemolysin (although he has found them to occur in its absence), and partly to the serum constituents, the nature and mode of which are as yet unknown. Kilduffe has offered experimental data confirming this. Our own findings, based on 500 samples of sera reveal but one anomalous reaction before and one after absorption. Such anomalous reactions do not, therefore, seem to depend upon the presence or absence of natural amboceptor. We believe, with Kolmer and Kilduffe, that the serum constituents play an important rôle in these reactions.

To judge the delicacy of any modification of the current complement-fixation test, clinical findings must be correlated with the laboratory reports. We set out to investigate the diagnostic findings of the 40 patients whose sera gave a positive reaction after absorption and a negative one before. The majority of these were latent syphilitics. Sera obtained from these at different intervals were consistent in giving inhibition of hemolysis after treatment of the sera, and noninhibition before. Kolmer and Steinfield[22]

propose as an explanation for false negative reactions on inactivated, untreated sera, that the blood of latent syphilitics contain little or no syphilitic reagin, at best, an insufficient quantity to fix the complement and specific antibody. Removal of the natural amboceptor, rendering the test more delicate, will aid in the laboratory diagnosis of these cases. It is our contention, therefore, that in spite of the additional time necessary for the absorption, the removal of the natural antisheep hemolysin is not only of theoretical, but of practical importance. We cannot hope to detect all cases of syphilis by the complement-fixation or any other test known today, but it is our duty to reduce the incidence of false negatives to a minimum by employing a test which will yield reactions of as maximum a degree of sensitiveness as is consistent with specificity.

Sera of probable latent syphilitics which contain large amounts of natural hemolysin and react negatively to the known complement-fixation tests of today, giving a further negative reaction after removal of the natural amboceptor, are believed by us to be not indicative of syphilis and will never be biologically so proved.

SUMMARY

1. Human sera frequently contain relatively large amounts of natural antisheep hemolysin. Antisheep hemolysin in sufficient quantities may serve as a source of error in the complement-fixation tests. An excess of natural antisheep hemolysin in human sera may yield falsely negative results, even though the sera may contain relatively large amounts of syphilitic reagin.

2. An appreciable difference in the readings of the complement-fixation tests are observed before and after the removal of natural antisheep hemolysin. The sensitiveness of the reaction is increased when the sera are "treated" and deprived of the amboceptor.

3. An occasional thermostabile anticomplementary activity is obtained with our method of absorption.

4. The Kolmer standardized technic for the complement-fixation test, in conjunction with hemolysin free sera, serves as a more delicate test than when used on simply inactivated sera.

5. The absorption of the natural antisheep hemolysin is most useful in the complement-fixation test on sera from latent syphilitics. It is in the cases of falsely negative results that special attention should be paid to measures which will not mask the syphilitic reagin.

6. In spite of the occasional anticomplementary reaction obtained after absorption, it is our opinion that "treatment" of all sera should be undertaken before the conduction of the complement-fixation test. A more reliable method for removing antisheep hemolysin from human sera should be looked for than that in current use.

I wish to express my gratitude to Dr. John A. Kolmer for suggesting and advising me upon this problem. To Dr. S. Greenbaum, I am indebted for supplying the clinical data. I am thankful to Doctor Dorothy Wilkes-Weiss for assisting me in the preparation of this paper.

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CHANGES OF IRRITABILITY IN WOMEN DURING THE MENSTRUAL CYCLE*

BY W. W. TUTTLE, PH.D., URBANA, ILL.

GENERAL observations seem to indicate that there is a variation in the general bodily tone in women during the menstrual cycle. This question has already been investigated by King,[1] Jacobi[2] and others. The data presented up to the present time seem to indicate that there is a period of hyperirritability immediately preceding the menstrual period which gradually returns to normal during the intermenstrual period.

The question arose in this laboratory, while making a comparative study of the irritability of men and women, whether it was necessary to consider menstruation in case of the women. In order to throw more light on this question it was decided to carry out the investigation herein reported.

Since it has been shown by the writer and numerous other investigators that whatever influences the general tone of a mechanism markedly affects the knee-jerk, it was decided to use this reflex as an index to any nervous change which might take place during menstruation. King[1] carried out an investigation similar to this and obtained results which, in general, supported those already obtained.

Data were collected from five women in good health ranging in age from twenty-five to thirty-five years. In all cases menstruation was regular and

*Department of Physiology, University of Illinois.
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normal, causing them no unusual discomfort. Each subject came to the laboratory at a definite hour daily during a complete menstrual cycle.

The cycle was divided into periods as follows[3]: (1) period of growth, five days preceding menstruation; (2) period of menstruation, approximately four days, or as reported by the subject; (3) period of regeneration, seven days; (4) period of rest, twelve days.

The experimental conditions were kept as constant as is possible in knee-jerk experiments. The subjects were adjusted in the apparatus[4] especially constructed for knee-jerk investigation and allowed to deliver jerks for twenty minutes. As soon as the adjustments were made, the operator left the laboratory and the subject remained alone throughout the experiment. The stimuli were delivered to the center of the ligamentum patellae at the rate of seven per minute. The exact point of impingement was carefully marked in each case in order to eliminate the possibility of varying the point of application of the stimulation from day to day. The forward component of the excursion of the foot is used as the index of the knee-jerk and is obtained by attaching the heel of the subject's shoe by a strong, inelastic cord to a stylus which writes on a smoked drum. The distance through which the stylus moves when the stimulus is delivered is referred to as the height of the knee-jerk.

Table I shows a comparison of the average height of the knee-jerks of each subject for each period during the menstrual cycle.

TABLE I

PERIOD	AVERAGE HEIGHT OF KNEE-JERK SUBJECT NO.				
	1	2	3	4	5
1	27.00	19.40	22.50	23.00	6.16
2	28.60	18.00	19.75	27.00	16.00
3	29.00	18.85	25.01	17.60	14.00
4	30.00	28.75	16.50	8.10	20.02

Subject 1 is an instructor in this department and is thirty years of age. She reports that ordinarily menstruation causes her no unusual inconvenience. Her records were made at 11:20 A.M. As shown by Table I there are no significant changes in the extent of the knee-jerk during the menstrual cycle.

Subject 2 is an instructor in the university and is twenty-three years of age. She reports that menstruation does not alter her physical condition as far as she is able to observe. The data presented in Table I were collected at 1:00 P.M. They indicate a hypoirritable condition during Periods 1, 2, and 3, followed by increased irritability during Period 4.

Subject 3 is a student twenty-five years of age. Her records were made at 8:00 A.M. The data in Table I indicate that Period 2 is preceded and followed by periods of hyperirritability. The subject is less irritable during Period 4. This subject volunteered no information concerning her observations with reference to the effect of menstruation on herself.

Subject 4 is twenty-four years of age and is an instructor in the university. Her records were made at 5:00 P.M. The data show that in her case there is a tendency toward increased irritability which reaches its maximum

during Period 2, gradually returning to normal during Periods 3 and 4. This subject reported that she felt more irritable during Period 2 than otherwise.

Subject 5 is an assistant in the university and is thirty-eight years of age. The data show that irritability is greatest during Period 4. Period 2 is preceded by a period of marked depression and is followed by a slight decrease in irritability.

Care was taken that the habits and activities of the subjects were as nearly uniform as possible for each day. Precautions were taken to prevent the operation of any of the significant factors which have been shown to affect the knee-jerk.

In observing the various subjects the writer noticed that, with but little exception, the records showed that disturbing factors had much more marked effect during Periods 1, 2, and 3 than during Period 4. This observation we believe is the most important point brought out in this investigation.

The data herein presented seem to indicate that the condition of the individual subject is a determining factor in the effect of menstruation. Some women will, no doubt, exhibit marked periods of increased and decreased irritability during the cycle in all cases; some will show this variation at different times, while others will show little or no effect. The investigations, up to the present time hardly justify any generalization with reference to the variation in irritability during the menstrual cycle.

The data presented in this paper, based on a very limited group of women, so far as they go show that: (*a*) there is, in some cases, no significant change of irritability during the menstrual cycle; (*b*) there may be a condition of depressed irritability during and preceding the menstrual period; (*c*) there may be a condition of hyperirritability preceding, during, and following the menstrual period. These findings support the conclusion, borne out by the general trend of recent investigations, that in case of healthy women, menstruation leads to no very significant disturbance of functions. Such variations as are noted are inconstant and inconsistent.

SUMMARY

While studying the change of irritability of five women during the menstrual cycle it was found that in one case there was no significant change in irritability; in one case, during Periods 2 and 3 there was a condition of depression; in one case there was a condition of hyperirritability during Periods 1 and 3; in one subject there was a condition of hyperirritability developed during Period 1, reaching a maximum during Period 2 and then gradually returning to normal during Periods 3 and 4; in one subject there was a period of marked depression during Period 1, followed by increased irritability in Period 2, gradually reaching a maximum during Periods 3 and 4. The data further show that any disturbing influences affecting the knee-jerk have a more marked influence during Periods 1, 2, and 3.

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REPORT OF A VERY SEVERE CASE OF JUVENILE DIABETIC COMA IN WHICH COMBINED TREATMENT WITH INSULIN AND BLOOD TRANSFUSIONS RESULTED IN PROMPT RECOVERY*

By Henry M. Feinblatt, M.D., and Irving Sherman, M.D., Brooklyn

BLOOD transfusions without insulin have been recorded in the treatment of diabetic coma, but the results have been mostly unfavorable. The justification for investigating the influence of transfusion upon the insulin-treated patient was furnished by the case of a moribund boy in profound dyspneic coma, who had failed to react favorably to the administration of large doses of insulin. Blood transfusion was then employed as a last resort. There was an instantaneous and very definite turn for the better, and the patient walked out of the hospital on the sixth day.

REPORT OF A CASE

The patient, a Jewish boy ten years old, was admitted in a state of coma to the United Israel-Zion Hospital, Sept. 18, 1924. The family history was negative. The patient had had measles, whooping cough, bronchopneumonia, tonsillitis and chorea. The tonsils and adenoids had been removed three years previously. The present illness began in the summer of 1923 with gradual loss of strength and weight and pain in the epigastrium. A physician found 5 per cent of sugar in the urine and a blood-sugar level of 170 mg. per 100 c.c. The boy was treated dietetically and with insulin, and the urine became sugar-free. In February, 1924, when insulin was discontinued for a time, the blood sugar promptly rose to 640 mg. per 100 c.c., and the urine contained 6 per cent of sugar. Treatment with insulin was resumed (8 to 10 units t. i. d.) and the blood sugar became normal and the urine sugar-free.

Unfortunately the boy fell into the hands of a quack, upon whose advice all insulin was discontinued on Sept. 16, 1924, and a liberal diet was allowed. On the following evening, he began to vomit and complained of great thirst and pain in the precordium and epigastrium. On Sept. 18, the patient passed into a comatose state and was hurried to the hospital.

Examination disclosed a sallow, poorly nourished boy, weighing 58 pounds, in deep coma. The temperature was 99.4° F., pulse 120, respiration 30. Breathing was of the Kussmaul type. The body and extremities were cold and clammy to the touch, the lips were cyanosed, and the alae nasi were widely dilated. There was a strong odor of acetone to the breath. The tongue was extremely dry and rough. The knee-jerks were absent. The skin of the extremities showed numerous needle points from previous injections of insulin and scars from old boils.

On admission, the urine had a specific gravity of 1028, and contained 3.3 per cent of sugar and a very large amount of acetone bodies. The blood sugar was 666 mg. per 100 c.c. The carbon dioxide combining power of the blood plasma, as measured by the method of Van Slyke and Cullen, was down to the extremely low figure of 7 volumes per cent. The red blood cell count was 3,600,000, hemoglobin 67 per cent (Sahli corrected). The white blood cells numbered 20,000, with 89 per cent polynuclears and 11 per cent lymphocytes.

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The boy entered the hospital at noon, and at 12:15 P.M. he received 35 units of insulin (H-insulin Lilly). At 12:30 P.M. a hypodermoclysis of 1,000 c.c. of salt solution was administered. At 2:30 P.M. 40 units of insulin were given, and at three o'clock 35 more units. The child came out of his coma, but would only open his eyes when disturbed and pushed.

At 3:30 P.M. 250 c.c. of blood were transfused from a healthy compatible donor by a direct vein-to-vein method. Within five minutes of this operation, there was a spectacular turn for the better. The patient became brighter, his eyes remained open, and he asked for water. The cyanosis disappeared, a good color returned to the cheeks, the pulse became stronger, and the respirations began to approach the normal type.

At four o'clock a hypodermoclysis of 800 c.c. of saline solution was administered. Thirty-five units of insulin were injected at 7:30 P.M., but the patient again appeared drowsy and the pulse was rapid and irregular. At 9:30 P.M. a second blood transfusion was done, injecting 250 c.c. of blood, and, as in the first instance, there was an immediate favorable response. The child brightened up and asked for water, and the pulse became stronger and more regular. Orange juice and water were allowed. Twenty-five units more of insulin were given at eleven o'clock. The patient was now out of coma and would answer when spoken to. When observed at 1:30 A.M., the boy was perfectly comfortable. His mental condition was clear and he asked for food and water.

At 2:30 A.M. the child complained of headache, and soon thereafter he began to sweat profusely. Symptoms apparently attributable to insulin shock were noted at 3:30 A.M. The boy suddenly awoke and became restless, constantly moaning. The pulse was of poor quality and respiration became of the Cheyne-Stokes' type. An intramuscular injection of 1 c.c. of adrenalin was given at 4:30 A.M., but breathing became deep and stertorous, and the patient began to foam at the mouth. Seven hundred cubic centimeters of saline solution were injected by hypodermoclysis, and at 6:30 A.M. the adrenalin was repeated. Oxygen was administered. The child rallied and by morning appeared very bright. On Sept. 19, at 9:30 A.M. the blood sugar was 110 mg. per 100 c.c. and the carbon dioxide combining power of the blood plasma was 50 volumes per cent.

From this time on improvement was rapid and progressive, and the patient was discharged from the hospital on Sept. 23, only five days from the time when he had been admitted in absolute coma. While in the hospital, he received 10 units of insulin three times a day, and the diet had a value of 15 gm. of carbohydrate, 25 gm. of protein, and 35 gm. of fat. At the time of discharge, the boy felt perfectly well, his urine was sugar-free, and his blood sugar was 111 mg. per 100 c.c.

When last examined on Dec. 1, 1924, the patient had no complaint, had not missed a day at school since Oct. 15, and played like a normal boy. He had gained 3 pounds, his general appearance, color, and muscle tone were good, and he was active and alert. The urine was not sugar-free but contained from 4 to 7 gm. of glucose in the twenty-four-hour specimens. Occasionally there was a trace of acetone and diacetic acid. The patient was on a diet of 60 gm. of carbohydrate, 65 gm. of protein, and 70 gm. of fat. He was receiving 40 units of insulin a day, 20 units before breakfast and 20 units before the evening meal. It was not deemed desirable to attempt to render the urine sugar-free for two reasons: (1) To accomplish this end by still further reduction of the diet seemed unwise in view of the state of the patient's nutrition; and (2) the fact that on several occasions there had been manifestations of insulin shock necessitated great care in adjusting the dosage of this agent.

COMMENT

Blood transfusion was tried in the treatment of diabetic coma before the days of insulin, but the results were mostly unfavorable. It has generally been held that the presence of diabetes mellitus is a contraindication of this procedure. Raulston and Woodyatt,[1] in 1914, reported a severe case of diabetic acidosis, in which the transfusion of 500 c.c. of peripheral venous blood from a healthy male donor had a decided effect for the worse on the

metabolism of the patient, as evidenced by a marked rise in the output of sugar, ammonium and acetone bodies and an increase in the glucose: nitrogen ratio. The patient died in typical dyspneic coma. Ottenberg and Libman,[2] in 1915, employed blood transfusion in four severe cases of diabetes mellitus, but failed to observe any benefit from this procedure. Three of the patients were in diabetic coma, while the fourth patient was a young woman with both diabetes mellitus and pernicious anemia. In no case did the transfusion have more than a transient effect on the coma. Garbat[3] treated a patient with diabetic coma by means of venesection and the removal of 500 c.c. of blood followed by blood transfusion by the citrate method. The coma had been of sudden onset following an operation for gangrene of the foot. There was distinct temporary improvement; the patient revived from her coma, but relaxed again in twenty-four hours. Venesection and transfusion were repeated, but, while a noticeable improvement again occurred, the patient did not recover.

Animal experiments have offered greater encouragement as to the efficacy of blood transfusion in diabetes mellitus than have the actual clinical trials. Carlson and Ginsburg,[4] in 1915, transfused dogs rendered diabetic by pancreatectomy with blood from normal dogs. The amount of blood transfused was approximately one-tenth that of the volume of the recipient's blood. As a result of this procedure, these workers observed a temporary (lasting four to eight hours) but definite and consistent lowering of the hyperglycemia and glycosuria. When diabetic dogs were used as donors, there was no effect on the hyperglycemia.

Notwithstanding this lack of encouragement from the literature, it was felt that the greatly enhanced metabolic capacity conferred by the administration of large doses of insulin might enable the diabetic to utilize an increment of fresh blood and thus to increase the level of oxidation, relieve dehydration, and enrich the buffer value of the blood. In a suboxidative disease such as diabetic coma, one would certainly expect a substantial addition of oxygen-carriers to be a potent factor leading to recovery. Acting upon the assumption that the earlier reported unfavorable results from blood transfusion in diabetes mellitus were largely attributable to the incapacity of the metabolism to handle the added burden of the transfused blood, and believing that the great aid to carbohydrate combustion rendered by the administration of insulin would remove this danger, we decided to try combined insulin therapy and blood transfusion as a last resort in this extremely severe case of diabetic coma.

Of course, it is realized that conclusions as to the efficacy of any therapeutic procedure based on the result obtained in a single case are not justified, but in this instance, the favorable response to blood transfusion in the insulin-treated patient was so instantaneous and definite as to strongly suggest that the transfusion played an important part in the patient's recovery. Any attempt in the present case to distinguish between the good which resulted from the insulin and that which could be attributed to the blood transfusion would be impossible.

SUMMARY

In the case of a comatose boy, aged ten years, with clinical and laboratory evidences of severe diabetes mellitus and maximal acidosis, large doses of insulin did not appear to yield marked benefit. A transfusion of 250 c.c. of unmodified blood in this insulin-treated patient, however, resulted in instantaneous and very definite improvement and the boy immediately came out of the coma. A second similar transfusion six hours later was again followed by striking betterment, and the patient walked out of the hospital on the sixth day. Although reports show that blood transfusions in diabetic patients without insulin have yielded mostly unfavorable results, the striking benefit noted in the present case is strongly suggestive of the possibility that blood transfusion in conjunction with large doses of insulin may prove to be a very valuable procedure in the treatment of diabetic coma.

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LABORATORY METHODS

A MICRO-FOLIN-WU METHOD OF QUANTITATIVE BLOOD-SUGAR ESTIMATION, USING 0.1 C.C. OF BLOOD*

By T. L. Byrd, M.D., Milwaukee, Wisconsin

THE present-day methods available for collecting blood specimens for sugar determinations are not applicable to all patients, and any suggestion of improvement over methods now in use will be welcomed by physicians and patients, where simplicity and accuracy are the ultimate ends.

It is obvious to all medical practitioners that a simple, accurate micro-method of quantitative blood-sugar estimation is needed; one which requires a minimum quantity of blood that can be obtained from finger or ear by a prick of a pin, under aseptic precaution; one which eliminates the fear, pain, and danger of a venepuncture, and saves time and effort for the operator; a method applicable to infants, small children, and obese individuals, from whom it is almost an impossibility to obtain blood for analyses, for reasons well known. With the unlimited use of insulin, a method with these advantages is necessary.

Within the past few years several attempts have been made, and micro modifications of the Folin-Wu method have been devised and published by Pollock and McEllroy,[1] Haden,[2] Baum and Isaacson,[3] Kramer and Gittleman,[4] Randles and Grigg,[5] and a number of micro-Lewis-Benedict methods. All micromethods heretofore described are modifications of the original Folin-Wu method, and necessitate variations in technic, which render them impracticable from a standpoint of simplicity and accuracy, for practical routine use.

The Folin-Wu method of analysis is one of the best now in use; when 1 volume of blood and 7 volumes of distilled water are mixed, complete hemolysis occurs; 0.1 c.c. of blood, plus 0.7 c.c. of distilled water plus 0.1 c.c. of 10 per cent sodium tungstate plus 0.1 c.c. of two-thirds normal sulphuric acid, precipitated and centrifuged at high speed for five to ten minutes, yields 0.7 c.c. of clear blood filtrate. Analysis of 0.5 c.c. and 2 c.c. of blood filtrate under similar conditions using solutions in proportion, will give the same end-results.

Evidently a device for collecting, measuring, and diluting 0.1 c.c. of blood, applicable to all patients is needed to perfect a micromethod of blood-sugar estimation. The idea was conceived of a blood diluting pipette, graduated at 0.1 c.c. and 0.8 c.c. respectively, with two chambers, the first having a capillary intake expanding into a fusiform shape of 0.1 c.c. capacity for obtaining and measuring the blood. The second having 0.7 c.c. capacity for

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diluting, mixing, and hemolyzing, with a constriction of the bore at each graduation. It is thus constructed to prevent the blood from flowing out, in case the operator has to pause to express more blood from the bleeding point; to insure accurate measurements; and so it can be held between the finger and thumb for mixing. The pipette is supplied with a detachable rubber tube and mouthpiece, and used in the same manner in obtaining and diluting blood as one for blood counting. This pipette solves the problem of obtaining blood from patients in small quantity. It is applicable to all patients, including infants, small children, and obese individuals. To those physicians in general practice, who have to collect blood and transport it to a laboratory, this instrument will be very useful, for the reason that a rubber band placed over the ends of the pipette after the blood is drawn and diluted, will enable it to be carried any distance without loss, and it will keep in this manner

TABLE I

TIME BLOOD WILL REMAIN PRESERVED AT ROOM AND INCUBATOR TEMPERATURE IN THE PIPETTE WITH A RUBBER BAND PLACED OVER THE ENDS

DILUTED WITH DISTILLED WATER

Blood No. 1 when taken 400 mg. after standing 9 hr. room temp. 400 mg.
Blood No. 2 when taken 400 mg. after standing 12 hr. room temp. 333.3 mg.
Blood No. 3 when taken 100 mg. after standing 24 hr. room temp. 0 mg.

DILUTED WITH 1:400 FORMALDEHYDE SOLUTION

Blood No. 1 when taken 285.7 mg. after standing 168 hr. (7 days) incub. 37.5° C. 285.7 mg.
Blood No. 2 when taken 250 mg. after standing 288 hr. (12 days) rm. temp. 250 mg.

TABLE II

ANALYSES OF 22 SPECIMENS OF BLOOD OF NONDIABETIC PATIENTS BY THE ORIGINAL AND THE MICRO-FOLIN-WU METHODS OF BLOOD-SUGAR ESTIMATION

ORIGINAL FOLIN-WU METHOD USING 2 C.C. OF BLOOD, OBTAINED BY VENEPUNCTURE			AUTHOR'S MICRO FOLIN-WU METHOD USING 0.1 C.C. OF BLOOD, OBTAINED WITH BLOOD DILUTING PIPETTE
No.	Name	Mg. per 100 c.c. of Blood	Mg. per 100 c.c. of Blood
1.	Mrs. I. R.	100	100
2.	Mrs. T. J.	100	100
3.	Mrs. E. M.	100	100
4.	Mr. O. R. T.	100	100
5.	Mrs. H. M.	100	100
6.	Mrs. J. H. M.	111	111
7.	Miss I. F.	111	111
8.	Mrs. W. B. B.	100	100
9.	Mrs. P. T. S.	100	100
10.	Mr. Wm. J.	100	100
11.	Mrs. B. B.	90.9	90.9
12.	Mrs. K. Z.	100	100
13.	Mrs. K. J.	90.9	90.9
14.	Mrs. A. C.	100	100
15.	Mrs. S. M.	100	100
16.	Mr. F. P. G.	83.3	83.3
17.	Mrs. M. S.	100	100
18.	Mrs. K. H.	100	100
19.	Mrs. P. C. M.	100	100
20.	Mr. J. L. Mc.	90.9	90.9
21.	Mrs. M. B.	111	111
22.	Mrs. K. S.	111	111

NOTE.—The colorimeter was set at the Number 10, the unknown of one technic was read; the standards changed, and the unknown of the other technic brought to the same figure as the former. (Whole numbers were used.)

from nine to twelve hours. In case it is necessary to send by mail, it can be diluted with 1:400 formaldehyde solution, instead of distilled water, which will preserve the blood for one week or more, at incubator (37.5° C.) or room temperature, without altering the sugar content. The Folin sugar tubes were reduced to one-fourth size, and other apparatus needed was supplied, thus minimizing, and not modifying the orginal technic in any single detail. A preliminary report with a drawing of the apparatus needed for the micro-Folin-Wu method, was published in the *Journal of the American Medical Association,* August 16, 1924.

Analyses were made on 86 specimens of blood under similar conditions, by the original and the micro-Folin-Wu method, without a single discrepancy. Tables II and III give the results obtained.

TABLE III

ANALYSES OF 28 SPECIMENS OF BLOOD OF DIABETIC PATIENTS BY ORIGINAL AND THE MICRO-FOLIN-WU METHOD OF BLOOD-SUGAR ESTIMATION

ORIGINAL FOLIN-WU METHOD USING 2 C.C. OF BLOOD, OBTAINED BY VENEPUNCTURE			AUTHOR'S MICRO FOLIN-WU METHOD USING 0.1 C.C. OF BLOOD, OBTAINED WITH BLOOD DILUTING PIPETTE
No.	Name	Mg. per 100 c.c. of Blood	Mg. per 100 c.c. of Blood
1.	Mr. J. R. F.	200 (a)	200 (a)
2.	Mr. J. R. F.	153.8(b)	153.8(b)
3.	Mr. J. T. M.	333.3(a)	333.3(a)
4.	Mr. J. T. M.	500 (b)	500 (b)
5.	Mrs. T. A. O.	181.8	181.8
6.	Mrs. McK.	133.3	133.3
7.	Mrs. P. A.	200 (a)	200 (a)
8.	Mrs. P. A.	142.8(b)	142.8(b)
9.	Mr. S. V. H.	125	125
10.	Miss M. D.	333.3	333.3
11.	Sr. M. M.	142.8	142.8
12.	Mrs. A. K.	400 (a)	400 (a)
13.	Mrs. A. K.	200 (b)	200 (b)
14.	Mr. G. B.	166.6	166.6
15.	Mr. L. P. K.	125	125
16.	Mrs. E. J.	142.8	142.8
17.	Mrs. S. J.	333.3	333.3
18.	Mr. J. E. P.	181.8	500
19.	Mr. H. F. D.	500	500
20.	Mr. F. R. F.	125.9	125.9
21.	Mr. T. A. C.	125	125
22.	Mrs. A. O. B.	142.8	142.8
23.	Miss H. A.	125	125
24.	Miss L. B.	125	125
25.	Mrs. C. P.	250	250
26.	Miss R. B.	250	250
27.	Miss E. S.	222.2	222.2
28.	Mrs. S. J.	200	200

NOTE.—All bloods were drawn and analyzed under the same conditions.

The data obtained from the sugar tolerance test is very valuable in cases of mild diabetes, renal diabetes, obesity, and pituitary disturbances. It is seldom done because it subjects the patient to several venepunctures at one-half and one hour intervals. This method removes every obstacle. Six tests were done using six specimens each, making a total of thirty-six specimens. Chart 1 gives the results obtained.

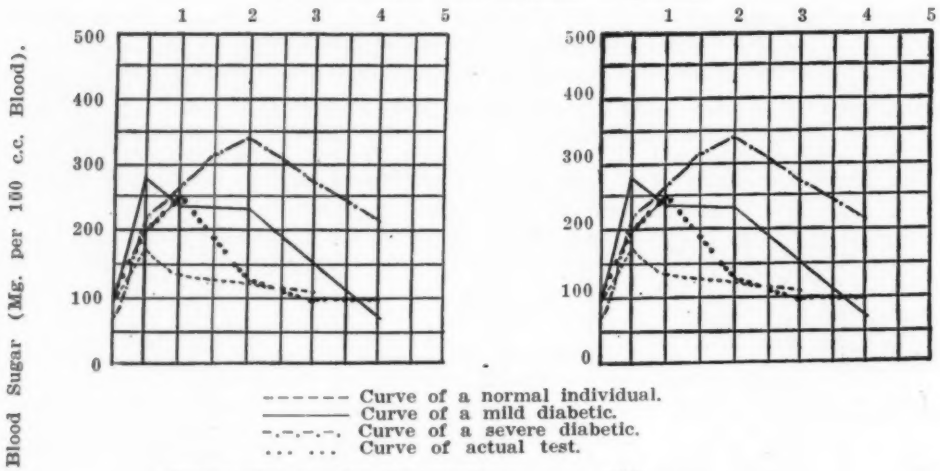
AUTHOR'S MICRO-FOLIN-WU METHOD

I. Method of obtaining blood from patient.

1. Rinse special blood diluting pipette with distilled water, alcohol, and ether; draw potassium oxalate solution, (5 to 10 per cent) to just above the 0.1 c.c. mark (expel the excess) to prevent the blood from adhering to the sides of the lower chamber of the pipette. (Where a number of bloods are to be taken with a single pipette, washing with distilled water, and the potassium oxalate solution will suffice as distilled water is the diluting fluid.)

2. Clean finger tip or ear with alcohol or iodine, prick with pin point or lancet, cleaned with the same drug, and from bleeding point, by means of blood diluting pipette, draw blood to 0.1 c.c. mark; wipe tip with a cloth or cotton, then emerse in distilled water and draw to 0.8 c.c. mark; shake well; complete hemolysis results.

CHART I



Grams glucose given by mouth .....100.....

Blood Sugar per 100 c.c.		Urine sugar per cent	
Fasting 100.0	Mg.	Negative	%
30 Min. 200.0	"	Negative	%
60 Min. 250.0	"	Slight reduction	%
2 Hr. 133.3	"	Slight reduction	%
3 Hr. 100.0	"	Negative	%
4 Hr. 100.0	"	Negative	%

Remarks ..... Normal Curve.

II. Precipitation of blood.

1. Transfer contents of pipette, (1 vol. of blood plus 7 vol. of distilled water) to a pyrex centrifuge tube.

2. Add 0.1 c.c. (1 vol.) of 10 per cent sodium tungstate solution; stir with glass rod.

3. Add 0.1 c.c. (1 vol.) of two-thirds normal sulphuric acid solution; stir with glass rod; thus precipitated, let stand from ten to twenty minutes.

III. Centrifuge at high speed from five to ten minutes. This yields 0.5 to 0.7 c.c. of clear filtrate.

IV. Method of sugar estimation.

1. Place 0.5 c.c. of blood filtrate in a minimized Folin sugar tube, graduated at 1 c.c. bulb and 6¼ c.c. total measurement.

2. Place 0.5 c.c. of sugar, standard solution, containing 0.2 mg. and 0.4 mg. per 2 c.c. respectively, in two other similar tubes.

3. Add 0.5 c.c. of alkaline copper solution to each tube.

4. Place in a water-bath and boil for six minutes.

5. Transfer to cold water-bath for from two to three minutes.

6. Add 0.5 c.c. of molybdate phosphate solution to each tube and let stand two minutes.

7. Add distilled water to 6¼ c.c. mark on each tube and compare in a colorimeter, the unknown to the standard, nearer comparing to the naked eye.

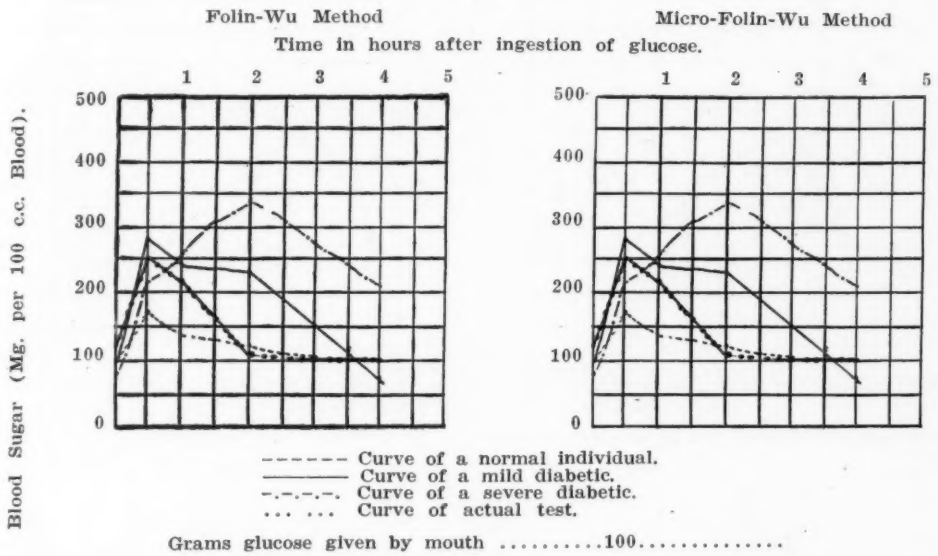
8. Calculation:

(Weaker) $\frac{S}{U} \times 100 =$ mg. per 100 c.c. of blood.

(Stronger) $\frac{S}{U} \times 200 =$ mg. sugar per 100 c.c. of blood.

NOTE.—The apparatus is washed with water. The pipettes are rinsed with the solution to be added, to avoid diluting.

CHART II



Grams glucose given by mouth .....100.....

Blood Sugar per 100 c.c.		Urine sugar per cent	
Fasting	125.0 Mg.	Negative	%
30 Min.	250.0 "	1.16	%
60 Min.	222.2 "	2.27	%
2 Hr.	111.0 "	0.65	%
3 Hr.	100.0 "	Negative	%
4 Hr.	100.0 "	Negative	%

Remarks ..... Renal diabetic.

PREPARATION OF SOLUTION

The solutions used are prepared the same as for the original, and in the following manner:

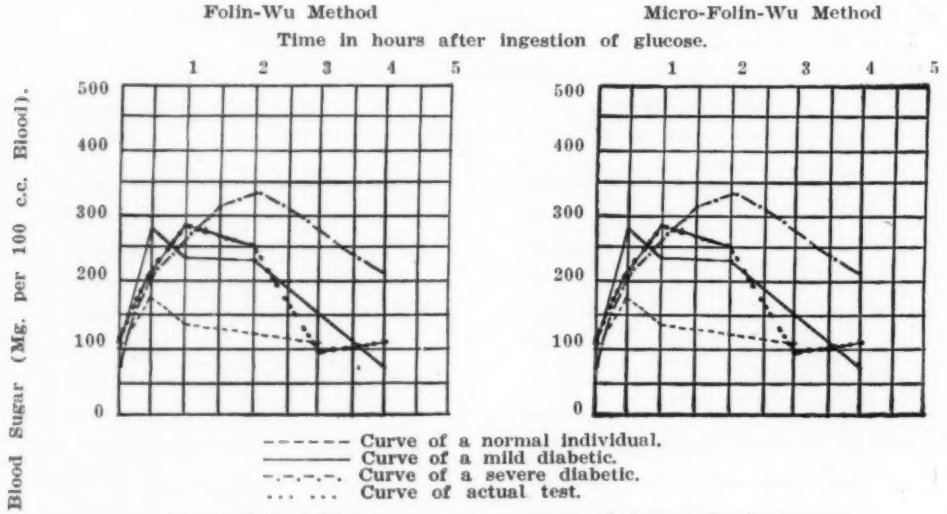
The standard sugar solution is prepared by dissolving 1 gm. pure anhydrous dextrose in 100 c.c. of saturated solution of benzoic acid. The working solutions are prepared by diluting 5 and 10 c.c. of the above stock solution to 500 c.c. with saturated solution of benzoic acid. These will contain 0.2 and 0.4 mg. dextrose per 2 c.c., respectively, and are fairly stable.

The alkaline copper solution is prepared by dissolving 40 gm. anhydrous

Na_2CO_3 in about 400 c.c. distilled water and placing in a liter flask. Add 7.5 gm. tartaric acid and when dissolved add 4.5 gm. crystallized $CuSO_4$; mix and make up to 1 liter. With impure carbonate, a sediment may form in a week or so; in such case, filter into another vessel.

The molydbate phosphate solution is prepared by putting 35 gm. molybdic acid in a one liter beaker. Add 5 gm. sodium tungstate, 200 c.c. 10 per cent NaOH, and 200 c.c. distilled water. Boil vigorously from twenty to forty minutes to remove NH_3; cool and dilute to 350 c.c. with distilled water. Add 125 c.c. 85 per cent phosphoric acid and dilute to 500 c.c. with distilled water.

CHART III



Grams glucose given by mouth .....100.....

Blood Sugar per 100 c.c.		Urine per cent	
Fasting	100.0 Mg.	Negative	%
30 Min.	222.2 "	1.25	%
60 Min.	285.7 "	3.57	%
2 Hr.	250.0 "	3.12	%
3 Hr.	99.9 "	Slight reduction	%
4 Hr.	111.1 "	Negative	%

Remarks .....Mild diabetic.

On account of the simplicity, accuracy, and many advantages of the micro-Folin-Wu method, it has been adopted as routine in our institution. The members of the staff order blood-sugar and sugar-tolerance tests on patients in the same manner as ordering a blood count, and all specimens are taken and transported to the laboratory by the technicians, in the same way, for the technic is the same. Up to the present time 100 specimens of blood have been analyzed and Tables IV and V will give results in a number of these cases.

CONCLUSIONS

This micromethod is a minimized and not a modified Folin-Wu method of blood-sugar estimation. It does not vary in a single detail from the original technic.

TABLE IV

ANALYSES OF 24 SPECIMENS OF BLOOD OF NONDIABETIC PATIENTS BY AUTHOR'S MICRO-FOLIN-WU METHOD, USING 0.1 C.C. OF BLOOD

NO.	NAME	MG. PER 100 C.C. OF BLOOD
1.	Dr. T. L. B.	90.9
2.	Miss M. F.	100
3.	Miss V. G.	100
4.	Mrs. J. D.	100
5.	Miss J. S.	100
6.	Miss H.	100
7.	Miss M. H.	100
8.	Miss E. C.	100
9.	Miss H. B.	100
10.	Mr. L. F. D.	83.3
11.	Miss L. A. E.	100
12.	Miss S. S.	90.9
13.	Mrs. J. McD.	100
14.	Mrs. J. O. C.	100
15.	Mrs. G. E. E.	100
16.	Mrs. S. G.	100
17.	Mrs. M. C. O.	100
18.	Mr. L. F.	90.9
19.	Miss W. W.	100
20.	Miss W. A.	100
21.	Miss K. D.	100
22.	Miss G. E.	100
23.	Miss W. W.	100
24.	Miss K. K.	90.9

NOTE.—Read on whole numbers.

TABLE V

ANALYSES OF 26 SPECIMENS OF BLOOD OF DIABETIC PATIENTS BY AUTHOR'S MICRO-FOLIN-WU METHOD, USING 0.1 C.C. OF BLOOD

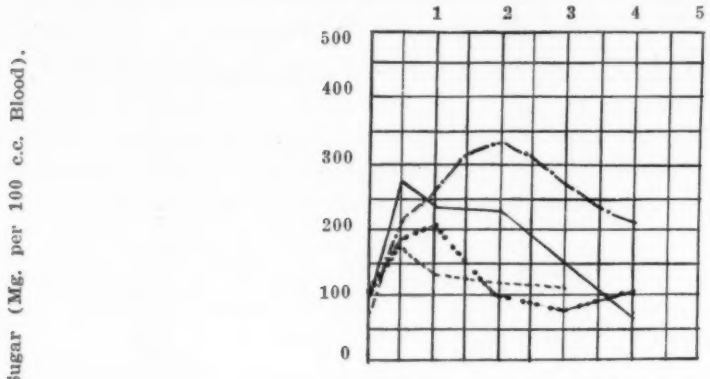
NO.	NAME	MG. PER 100 C.C. OF BLOOD
1.	Mrs. M. H.	333.3
2.	Mrs. S. S.	333.3
3.	Mrs. C. G.	400
4.	Mr. J. S.	200
5.	Mrs. A. S.	333.3
6.	Mr. J. K.	200
7.	Sr. M. B.	500 (a)
8.	Sr. M. B.	400 (a)
9.	Mrs. M. D.	400 (a)
10.	Mrs. M. D.	400 (b)
11.	Mrs. M. D.	400 (c)
12.	Mrs. M. D.	285.7(d)
13.	Mrs. M. D.	285.7(e)
14.	Mrs. M. D.	333.3(f)
15.	Mrs. M. D.	400 (g)
16.	Mrs. M. D.	333.3(h)
17.	Mrs. M. D.	285.7(i)
18.	Mrs. M. D.	181.8(j)
19.	Mrs. M. D.	166.6(k)
20.	Mrs. M. D.	142.8(l)
21.	Mrs. M. D.	133.3(m)
22.	Mrs. M. D.	166.6(n)
23.	Mrs. M. D.	142.8(o)
24.	Mrs. M. D.	153.8(p)
25.	Mrs. M. D.	111. (q)
26.	Mrs. M. D.	133.9(r)

Insulin Treatment

CHART IV

Micro-Folin-Wu Method

Time in hours after ingestion of glucose.



------ Curve of a normal individual.
———— Curve of a mild diabetic.
-.-.-.-. Curve of a severe diabetic.
... ... Curve of actual test.

Grams glucose given by mouth.....100.....

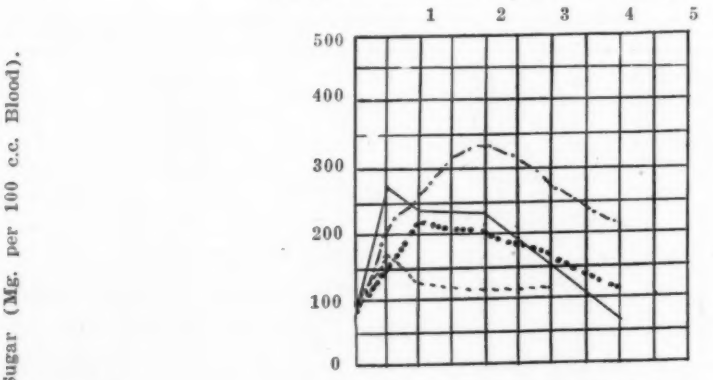
Blood sugar per 100 c.c.			Urine sugar per cent	
Fasting	100.0	Mg.	Negative	%
30 Min.	181.8	"	0.4	%
60 Min.	222.2	"	1.85	%
2 Hr.	100.0	"	1.35	%
3 Hr.	83.3	"	Slight reduction	%
4 Hr.	90.9	"	Negative	%

Remarks ..... Renal diabetic.

CHART V

Micro-Folin-Wu Method

Time in hours after ingestion of glucose.



------ Curve of a normal individual.
———— Curve of a mild diabetic.
-.-.-.-. Curve of a severe diabetic.
... ... Curve of actual test.

Grams glucose given by mouth.....100.....

Blood sugar per 100 c.c.			Urine sugar per cent	
Fasting	100	Mg.	Negative	%
30 Min.	133.3	"	Slight reduction	%
60 Min.	222.2	"	1.56	%
2 Hr.	200	"	1.92	%
3 Hr.	166.6	"	1.72	%
4 Hr.	125	"	0.56	%

Remarks ..... Mild diabetic.

The blood diluting pipette solves the problem of obtaining the blood in small quantities, and is applicable to all patients, including infants, small children, and obese individuals, in whom it is almost impossible to obtain blood by venepuncture. There is no danger of blood clotting in the pipette, because there is ample time to draw and dilute the blood, and this can be done by any technician who can obtain blood for counting, for the technic is the same; while venepuncture usually requires the skill of a physician. This pipette provides a simple means whereby physicians out in general practice can obtain, measure, and dilute specimens of blood and transport them to a laboratory, by placing a rubber band over the ends. Diluted with distilled water, it will keep from nine to twelve hours at room temperature. Using 1:400 formaldehyde solution as diluent, it will remain preserved for one week or more, at incubator (37.5° C.) or room temperature, without altering the sugar content in the least. This makes it possible to send specimens of blood in this manner any distance through the mail.

This method has distinct advantages over the original, because it eliminates the necessity of having on hand sterile syringes and needles, and test tubes containing potassium oxalate.

It saves the patient the fear, pain, and danger of venepuncture.

It saves time for the operator, since blood is drawn, measured and diluted in a few seconds' time.

Its simplicity and accuracy make it desirable for routine use in any institution, clinic, or private laboratory, and for these reasons I submit it to the profession, since in all scientific investigation, we should endeavor to subject our patients to as little pain as possible, and at the same time give accurate results.

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A SIMPLE AND SENSITIVE MODIFICATION OF THE WASSERMANN TEST*

By E. E. Ecker, Ph.D., Cleveland, Ohio

INTRODUCTION

SINCE the Wassermann test has become a widely employed laboratory procedure many attempts have been made to simplify the method, to render it more sensitive and accurate. As with any other laboratory procedure, familiarity with a given method produces a degree of confidence that is sometimes unjustified by the facts. Many attempts have been made in various countries to devise a standard method which can be adopted by a large number of laboratories. Throughout this country the labors of Kolmer[1] and his associates are well and favorably known and the modification they have proposed has been widely adopted. Our studies of the situation are in general agreement with those of Ottenberg[2] and Ottenberg and Wisler,[3] who find that the Kolmer modification is somewhat cumbersome, employs larger amounts of material than seems absolutly essential and fails to make provision for that antisheep amboceptor not uncommonly present in human serum. This is further complicated by the fact that the methods so far devised for eliminating the antisheep amboceptor in human serum are likely to increase the anticomplementary activity of that serum. It is difficult to compel all laboratories to adopt a uniform method, and admitting that this aim is desirable, widespread adoption should depend rather upon convincing evidence that a given method is the best so far available than by setting up a standard upon any particular group of appointed individuals. It is hoped that the method herein described will, with proper indication and control, meet the necessities of the present unfavorable situation. Its principle advantages are simplicity, avoidance of the interfering reaction due to the presence of antisheep amboceptor in human serum, and what is believed to be increased sensitivity without particular danger of false positives. The method employs a uniformly prepared antigen, five doses of hemolytic amboceptor and delicately titrated complement. It is well known that the phenomenon of hemolysis will occur in the presence of one unit of cell suspension, one unit of amboceptor and one dose of complement. It is equally well known that if the dose of amboceptor be increased, the dose of complement can be reduced. Furthermore, if a test of this sort be set up with four units of hemolytic amboceptor, one dose of cell suspension and a carefully titrated amount of complement, the addition of one or two units of amboceptor derived either from a specific immune serum or from human serum, the outcome is not demonstrably altered. These are the principles underlying the proposed

*From the Department of Pathology, School of Medicine, Western Reserve University, Cleveland, Ohio.
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modification which in seven years of practice, has given convincing evidence of simplicity, sensitivity and accuracy.

In the report of the hygienic committee of the League of Nations given by Sachs,[4] it is pointed out that there is no advantage in employing more than one antigen in these tests. The method of preparation of antigen given in this paper has been constantly successful. It has a high titer with low anticomplementary and no hemolytic activity. It is an acetone insoluble antigen and for routine work requires no cholesterol or other substance to increase its binding power.

The method of titration of complement should be most carefully followed. Ice box fixation has proved to be of the utmost value. As the result of numerous tests with this method it has been found, as pointed out by Kline, Applebaum and Lundagen,[5] that overnight ice box fixation is distinctly preferable to four hour fixation, because it increases the sensitiveness of the test and permits the recognition of partial fixation in treated and doubtful cases. The principles embodied in this communication have been followed in numerous hospitals in the city of Cleveland with the utmost satisfaction.

PREPARATION OF MATERIALS

Antigen.—The antigen as now employed is a modification of Ecker and Sasano's antigen.[6] The Bordet and Ruelens[7] antigen may also be used with success. The new antigen as prepared by the author is as follows: Extract in a 500 c.c. Erlenmeyer flask, with attached coil condenser over a water-bath, 30 grams of finely powdered, rapidly dried, mixed calf or beef hearts with 200 to 300 c.c. of C. P. acetone for a period of three to four hours. Avoid overheating. Remove from water-bath and chill mixture in ice box overnight. Filter through paper and allow acetone to evaporate entirely in an incubator at 37° C. Extract the acetone-free dry powder in the same apparatus with 200 to 300 c.c. of C. P. 95 to 100 per cent methyl alcohol (acetone-free) for three to four hours. Filter and evaporate over water-bath to about 90 c.c. Titration of this antigen should be performed with strongly positive, weakly positive and negative serums, using dilutions of 1:2, 1:4, 1:8, etc., in 0.1 c.c. doses of each dilution. Nonspecific inhibition may occur in a 1:8 dilution or even higher but these extracts are powerful and not hemolytic. It may be cholesterolized if required with 0.2 per cent cholesterol. Because of precipitation, heat extracted antigens should be warmed prior to use. The antigen can then be diluted directly with physiologic saline. The dose employed is 0.1 c.c. of a dilution at least three times its inhibitory value. Titration of the antigen with a two-plus and three-plus serum is of great importance.

Patient's Serum.—The patient's serum is poured off from the clotted blood and centrifuged. The clear serum is pipetted off and inactivated at 55° C. for 20 to 30 minutes, the shorter period being less destructive of the "reagin."

Complement.—Pooled guinea pig serums are used, secured by bleeding from the heart or carotid as described by Karsner and Ecker.[8] For titration one c.c. of the serum is diluted with 29 c.c. saline using 0.03, 0.06, 0.09, 0.12, 0.15, 0.18, etc., in test tubes containing 1 c.c. or 0.5 c.c. of sensitized sheep corpuscles (to be

described later) and balanced with saline. The mixture is well shaken and incubated in the water-bath at 37° C. for one-half hour. The amount in the first clear tube, free from shadows and sediment, constitutes the unit. For use in reactions the remainder of the serum is diluted 1 : 10, and 2 units employed in the overnight fixation method.

Cell Suspension.—Wash 10 c.c. of whole defibrinated sheep blood four times with saline. Dilute the residual cells to 200 c.c. with saline or to 100 c.c. if only 0.5 c.c. sensitized cell suspension is to be employed. Standardize the suspension by determining the hemoglobin content with the Sahli method or by Hopkins tube as described by Ecker and Rees.[9]

Amboceptor.—The presence of natural antisheep hemolysin has been a matter of considerable difficulty in the hemolytic system because of its inconsistency (Kalisky,[10] Morgenroth and Sachs,[11] and Noguchi[12]). Noguchi for this reason chose an antihuman system. In an unpublished study Sasano and Ecker found that 19 per cent of serums contained two or more units of natural hemolysin and 19.6 per cent a noticeable amount but less than two units, while rarely serums containing four or more units are encountered. The Bauer technic[13] utilizing the natural amboceptor, has been discarded (Hoehne,[14] Meirowsky,[15] and Swift[16]). Cummer and Dexter[17] conclude that it is essential to take notice of presence or absence of this natural antibody in the performance of the test. Similarly Seelman[18] sees a source of error in the complement-fixation test in which an antisheep hemolytic system is employed. Simon[19] suggests the use of Noguchi's antihuman hemolytic system or preliminary extraction of the natural antibody. Kolmer and his collaborators, however, advocate the use of two units of amboceptor while Kahn[20] removes the natural hemolysin.

If the amount of amboceptor in the hemolytic system be large, not only is the required amount of complement reduced, but moderate increases in the quantity of amboceptor do not influence the reaction in any significant degree. This well established principle is a most important feature of the method here outlined. Those who have employed it (McKenzie,[21] Field,[22] Kaup,[23] Ranque and Senez[24]), have not sufficiently emphasized its value in the Wassermann test. Thiele and Embleton,[25] in a study of the mechanism of antibody action, have shown that increasing amboceptor diminishes to a certain degree the complement concentration necessary to produce lysis. Above a certain amount, increase in the quantity of amboceptor does not diminish the necessary complement concentration. It, however, greatly accelerates the rate of the reaction. Recently, Hill and Parker,[26] in agreement with Thiele and Embleton, pointed out that the main hemolytic reaction proceeds according to the law of unimolecular reactions and that the rate of reaction is, within certain practical limits, proportional to the square root of the amount of amboceptor per cell. The effects of increasing the amboceptor is, therefore, first to increase the "titer" of the complement, and second to increase the rate of the reaction. With increasing amboceptor the first effect reaches its limits earlier than the second. They showed that the complement concentration for complete hemolysis falls sharply with the first increments of amboceptor, but increases beyond about 4 or 5 M. H. D. produce no further effect in this respect. Hill, McKinstry and

Parker[27] in a recent modification of Harrison's method use 8 M. H. D. of amboceptor to sensitize their cells. Sasano and Ecker in a study of over 3,000 serums have found that with a well-balanced hemolytic system, an addition of as many as twenty units of amboceptor does not produce a complete hemolysis of one dose of sheep cell suspension, in a given time, when less than one minimum complement unit is present. Titrations of complement with cells sensitized with five units of amboceptor will completely eliminate the factor due to natural hemolysin, thus obviating the employment of an antihuman system or removal of the natural antibody. It was found further that in certain cases serums from which the natural antibody had been removed become more or less anticomplementary or weaker and some even negative. Again the use of five units of amboceptor does not in the least sacrifice the sensitiveness of the test and has the added advantage of a minimum quantity of complement. The amboceptor is prepared in the usual way, but bleedings are made five days following the last injection. The serum is inactivated at 55° C. for one-half hour and preserved with 50 per cent C. P. glycerol. It should be kept in an ice box, titrated often and have a titer of at least 1:1000. In titration, 0.5 c.c. of each dilution is added to 0.5 c.c. of the standardized cell suspension and 0.25 or 0.5 c.c. of pooled complement. In the reactions five units should be used.

Sensitization of Cell Suspension.—The necessary amount of amboceptor is added to 200 c.c. of saline and shaken. This solution is then added to 200 c.c. of the prepared erythrocytes and again shaken. If less than 1 c.c. (0.5 c.c.) of the sensitized cells are to be used, the cells may be suspended in 100 c.c. saline, the necessary amounts of amboceptor in 100 c.c. saline added, and the two mixed. The mixture is allowed to stand in a water-bath at 37° C. for thirty minutes prior to titration of complement. The writer uses 1 c.c. of first mixture.

METHOD

The technic is essentially the three tube system of Citron. The patient's serum is used in doses of 0.05 c.c. and 0.1 c.c., to which is added the antigen and complement. The control tube for patient's serum contains 0.1 c.c. serum plus complement and salt solution to balance. Two antigen control tubes contain respectively 0.1 c.c. and 0.2 c.c. of antigen dilution, together with complement and salt solution to balance. Controls should be set up with a known negative, a known two or three-plus positive and a four-plus positive serum, together with antigen and complement. The tubes are shaken and allowed to stand for five to fifteen minutes. Complement is then added in doses of two units and the tubes placed in an ice box at 4° to 8° C. overnight. The sensitized cell suspension is also kept in the ice box. The next morning the tubes and the cell suspension are warmed and the latter added to the tubes. The mixture is incubated for thirty minutes at 37° C. Readings are made at once and after sedimentation. The readings can be made with the aid of the Citron scale.

Spinal Fluid.—If the fluids be clear no inactivation is necessary. If they be bloody, they are centrifugalized and inactivated for fifteen minutes at 55°

C. If the quantity of fluid be sufficient, doses of 0.1 c.c., 0.25 c.c. and 0.5 c.c. are employed, and similar amounts are used in the controls. Experience has shown that with this method and overnight cold fixation, a maximum of 0.5 c.c. spinal fluid is adequate. Antigen dilution is employed in doses of 0.1 c.c. As with serum two units of complement are used. Saline is added to balance.

Quantitative Reaction.—This can be done by dilution of the patient's serum.

CONCLUSIONS

The method as described is not only highly specific, but has been shown by clinical correlation to be extremely sensitive, especially in relation to the treated cases. No false positive reactions have been recorded in examination of the serum of a large number of cases of scarlet fever and tuberculosis, as well as many normal pregnant women. Of particular interest is the case of malaria reported by McConnell[28] in which a positive reaction was obtained by this method, in spite of complete absence of clinical findings indicative of syphilis. At necropsy, however, there was an outspoken syphilitic aortitis with involvement of the aortic leaflets. As further refinements are made in the preparation of materials they can be adapted without essentially altering the basic principles of this method.

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A NOTE ON THE USE OF SOLID MEDIA FOR THE DETECTION OF GAS PRODUCTION BY BACTERIA. I*

By L. O. Dutton, M.S., and Velma M. Rutherford, A.B., Memphis, Tenn.

IN a recent article, I[1] pointed out that there was a source of error in the detection of gas production by bacteria on agar-agar if the medium was allowed to dry to a certain extent. On agar tubes that had been kept in the ice box for a period of fifteen days or more, certain of the tubes failed to show gas bubbles when inoculated with a known fermenter of the sugar used. This result was thought to be due to the drying and resulting increase in the viscosity of the agar. It was suggested that to overcome this defect the media be stored in a humidor. An experiment was reported showing that media stored in such a container retained its ability to detect gas longer than the control medium stored in the ice box.

Having tried to overcome, practically, the too rapid drying of agar by storage in a very tight ice box in which the moisture content was maintained as high as possible, without success; and having met difficulties in the use of an efficient humidor large enough to be of service, some more simple manner to solve the problem was sought. It was thought that remelting the medium in boiling water and reslanting immediately before use might lower the viscosity to a point comparable with freshly made agar, and thus restore the efficiency of the medium for detecting gas production.

This was found to be the case, and upon trial, agar of an old batch revealed gas production readily. It remained only to prove that media stored in the ice box and then remelted, would give constant results in this regard. To arrive at the value of the method, a simple comparative experiment was done.

A number of tubes of dextrose agar were prepared with bromcresol-purple as the indicator of acid production. They were sterilized at twenty pounds pressure for twenty minutes. Immediately after slanting and cooling, ten of the tubes were planted with a culture of B. paratyphosus B. This was for the purpose of arriving at a more or less rough idea of the amount of gas to expect in the amount of medium employed, and to determine the limits of variability in this regard. The results were recorded in number of gas bubbles formed in the medium. Of course such a method is open to many objections but it is surprising how constant the number of bubbles will remain in a series of tubes, and how narrow will be the limits between which this number varies. The average number of bubbles produced in the ten tubes was fourteen and in each the medium was raised from the bottom of the tube. The largest number of bubbles recorded was seventeen and the smallest twelve. With this as a

*From the Laboratories of the Methodist Hospital, Memphis, Tennessee.
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[1]Dutton, L. O.: A Note on Certain Limitations of the Use of Solid Media for the Detection of Gas Production by Bacteria. Jour. Lab. and Clin. Med., September, 1924, ix.

basis, a series of cultures was made, inoculating two tubes, one of which had been freshly remelted and slanted on successive days. The results in regard to gas detection are shown in Table I.

It is clear that the remelted agar retained its ability to detect gas while the unmelted agar lost that quality almost completely. The striking difference between the two sets of media is not fully reflected by the number of gas bubbles alone. At no time during the twenty-eight days did the remelted agar fail to show a space between the bottom of the agar and the bottom of the tube. In contrast with this, the unmelted agar failed to show such a separation after the sixth day except for four days at irregular intervals. The small number of bubbles recorded in the older cultures on the unmelted agar were along the line of stab and in a study of unknowns would have been recorded as doubtful results. It is doubtful, in the light of past experience, whether the unmelted agar would have shown any bubbles at all, had the inoculations been made without stabbing.

The simple precaution of remelting and reslanting agar tubes that are to be used for the detection of gas production seems, then, to assure constant and accurate results.

In laboratories where large numbers of fermentation studies are done, there is probably a rapid enough turnover of the media stock to obviate the necessity of such a procedure. In those laboratories in which fermentation studies are done less often, however, there is a definite need for some method of keeping solid media that is to be used for gas detection in a fresh state. Errors in classification of species have come to our attention frequently, where the difficulty was traced to the failure of solid media to show gas. In our own laboratory several "new species" and "atypical variants" have turned out to be old friends, when care was taken in the fermentation studies.

Our conclusions are that there is ample opportunity for error in gas detection on agar by allowing it to be stored several days before use; and that in the interest of economy, both of time and materials, as well as the reliability of results, a procedure that will forestall such errors and at the same time allow the use of old media is desirable. Such a method is suggested, remelting just before use, and a simple experiment is detailed to prove that the method will prove efficient in practice.

TABLE I

TABLE SHOWING THE NUMBER OF GAS BUBBLES IN AGAR TUBES OF VARYING STORAGE PERIODS UP TO THIRTY DAYS. THE INOCULATIONS WERE MADE EACH DAY WITH B. PARATYPHOSUS B ON A REMELTED AND AN UNMELTED TUBE

Age of agar in days	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
No. of bubbles in remelted tube	8	19	15	14	12	8	12	7	16	8	9	17	13	17	16
No. of bubbles in unmelted tube	10	11	10	11	9	18	5	12	17	2	1	2	9	15	4
Age of agar in days	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
No. of bubbles in remelted tube	16	16	12	17	15	11	12	14	13	14	16	15	17	14	13
No. of bubbles in unmelted tube	13	3	3	3	1	4	1	3	2	2	1	1	1	1	1

AN ELECTRODE FOR MEASUREMENTS OF SKIN POTENTIAL*

By Walter C. Alvarez, M.D., B. L. Freedlander, M.D., and L. B. Clark, San Francisco, Calif.

IN 1882, Hermann[1] connected different parts of seedlings to a delicate galvanometer and found that the rapidly growing tips of the leaves and of the roots were negative (through the galvanometer) to the cotyledons. In recent years Child[2] and his associates have been studying similar gradients of potential along the axes of small organisms and have shown that they correspond to gradients of metabolic activity. Potential differences exist also between the various parts of the human body, but so far as we can find, no

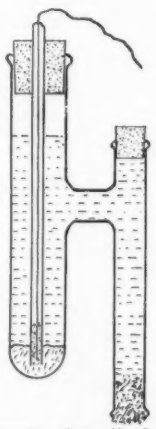

Fig. 1.—Electrode described in the text.

attempt has ever been made to map them. It has long seemed to one of us that such a map might be worth making as it might throw light on the origins of the skin current. Relics of axial gradients might be looked for but we would hardly expect to find them in so complicated and so thoroughly integumented a structure as the human body.

As so often happens in research work, the little problem which was to take weeks took months and then ended in discouragement. Our only excuse for publishing this short note is that most of our labor went into securing a satisfactory electrode: one which we believe can now save other travelers along this road from much vexation and wastage of time. The difficulty which we found with all the various nonpolarizable electrodes, with zinc—zinc sulphate, mercury-calomel, ferric-ferrous sulphate, and other combinations, was that after a little contact with the skin they developed potential differences which made them too erratic for use. Success came finally through

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following a suggestion of Lund[3] who we found had just been through similar difficulties. We wish to thank him for his help so freely given. Our electrode which is depicted in Fig. 1 is a modification of his. In the bottom of the larger tube was placed a small amount of amalgam made up of 3 per cent lead and 97 per cent mercury. The smaller tube dipping into the lead contained a copper wire which was stuck into a small amount of stiff amalgam made of finely divided copper and mercury. The contact with the skin was made through a gauze wick which could easily be renewed together with the solution in the little side arm.

These electrodes remained equalized over long periods of time and when, occasionally, they did develop differences in potential we could quickly restore the balance by shorting them for a few minutes in a beaker of the

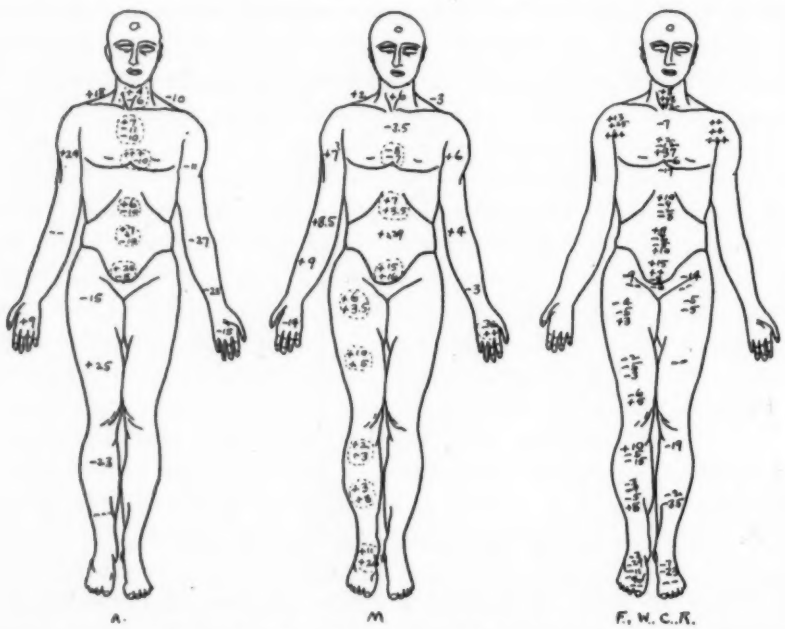

Fig. 2.—*A* and *M* show the data from two subjects. See text. The figures enclosed in circles represent measurements in the same place on different days. *F, W, C, R,* represent data from four subjects. The signs −− or ++ indicate deflections beyond the galvanometer scale.

lead chloride solution. The readings were always made in duplicate with the positions of the electrodes reversed between times. When these readings were equal and of opposite direction we could be pretty sure of our results. A highly sensitive Leeds and Northrup d'Arsonval galvanometer was used.

Fig. 2 shows the type of chart which we have obtained. The readings are in arbitrary units of the galvanometer scale, and all are relative to the potential of a spot in the middle of the forehead. A double plus or double negative sign means that the deflection was too wide for the scale. When two or more figures are given in a circle they denote readings obtained on different days. The third chart in Fig. 2 shows data obtained from four subjects. These charts are not as complete as one might wish but even with

the electrodes working well it takes a good deal of time to be sure of a few measurements.

When it was found that these readings could be duplicated closely at intervals during any one session of a few hours it seemed as if they must be indicative of local peculiarities of some sort, but unfortunately they varied so markedly on subsequent days and in different subjects that we soon despaired of getting any definite information, and it seemed best to wait until more could be learned about the exact mode of production of the potential differences. A review of the literature shows that most of the work on this subject has been done by the experimental psychologists who have tried to find out why larger differences suddenly appear when the subject experiences a strong psychic stimulus. An excellent review of that work has been written by Prideaux.[4] Some of the more recent studies are by Gildemeister,[5] and Albrecht.[6]

Naturally, one of the first questions that arises is: how much of the current depends upon local differences in the intensity of perspiration? A glance at Leva's[7] charts shows that the sweat glands are most concentrated on the palms of the hands and the soles of the feet. There are large numbers also on the forehead and in the axilla. They are but few on the arms, thighs, legs and back. This distribution agrees with the findings of Waller[8] who, with the help of little watch crystals containing calcium chloride, measured the amount of perspiration in the different regions.

In our studies, we did find a good deal of correlation between the intensity of perspiration and the amount of current, but unfortunately, the regions of heaviest perspiration were sometimes positive and sometimes negative to the forehead, all of which is hard to understand. Furthermore, big potential differences could often be obtained between two places like the cheeks where there are few sweat glands. A careful washing of the skin made no change in the results. Warming the skin where one of the electrodes was to be applied had very little effect on the intensity or direction of the current.

SUMMARY

An electrode is described, suitable for studies on the skin current.

An attempt has been made to map the differences in potential in different parts of the surface of the body. The results so far are too erratic to give us much information as to the origin of these differences.

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A NEW USE FOR A VALENTIN KNIFE*

By William Snow Miller, M.D., Madison, Wis.

SOME two generations ago, before the advent of the microtome, the two-bladed knife of Professor Valentin was much used in obtaining thin sections for microscopic study. This was, in the hands of an expert, an efficient instrument for obtaining single sections.

The knife was made in two forms. The original form consisted of two blades, which were supposed to be capable of being so separated that the blades were parallel to each other; the distance between the blades being regulated by a screw (Fig. 1). When well made it was a serviceable instrument; otherwise a most unsatisfactory one. This form was later improved

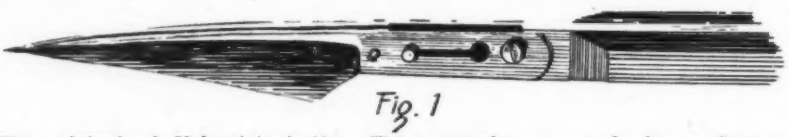

Fig. 1.—The original of Valentin's knife. This example was made by a German cutler whose name has been obliterated through use.

Fig. 2.—The improved form devised by Matthews. This particular knife was made by a Philadelphia cutler whose name was obscured when the knife was repolished.

by the addition of a second screw by means of which a more exact parallelism between the blades could be maintained.

An improved form was devised by an Englishman by the name of Matthews (Fig. 2). In this form the blades could be entirely separated from each other; a very desirable feature when it came to sharpening the blades and to cleaning them after use.

After more than a year's search I was able to resurrect an example of each type of the knife. Preference is given to the form illustrated in Fig. 2.

For several years I have been studying tuberculosis as it occurs in the human lung, and as it is produced experimentally in the lungs, and other organs, of the rabbit and guinea pig. I found it difficult to cut out blocks, with parallel sides, from a lung which contained very young tubercles, and not to include so much of the surrounding tissue as to make the cutting of a tubercle into serial sections and the mounting of them, a tedious task.

The idea came to me that a Valentin knife might be advantageously

*From the University of Wisconsin, Madison, Wisconsin.
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used for this purpose. I had used one in my student days, 1876-1879, but I did not own one.

I wrote to various dealers asking if they still kept in stock such a knife, and during vacation trips I visited instrument dealers in various parts of the country and asked the same question. In most instances I met with absolute ignorance of the existence of such a knife; by a few I was told they had discontinued making them. Finally I obtained from Professor Gage, of Cornell, the knife shown in Fig. 2, but in so dilapidated a condition that he feared it could not be made usable. However, it proved to be made of excellent steel and is now as good as new. Later I obtained from Pennock, of Philadelphia, the example shown in Fig. 1; but this proved to be so ill made that its use is limited.

I have found the knife illustrated by Fig. 2, exceedingly useful in cutting out blocks, with parallel sides, from one to five millimeters in thickness. If the edge be of the requisite keenness, it can be used on fresh lungs; but it works much better after the lung has been fixed and hardened, and this is the time when I make the most use of it. On the more solid viscera, as the liver or the kidney, it can be used advantageously any time.

In my hands the knife has proved so useful that it seems to me there is still a place for a Valentin knife in the histologic or pathologic laboratory, even though it be no longer useful in the work for which it was originally designed.

METHOD FOR CLARIFYING CLOUDY URINES FOR THE PHENOLSULPHONEPHTHALEIN TEST*

By E. A. Hewitt, D.V.M., St. Paul, Minn.

THE clinical determination of renal function by means of phenolsulphonephthalein was devised by Rowntree and Geraghty and depends upon the injection into the tissues of a dyestuff which is eliminated rather rapidly by the normal kidney, and can be estimated quantitatively in the urine.

The technic usually employed is as follows:

1. Give the patient 300 to 400 c.c. (about two glasses) of water to promote secretion of urine.

2. Twenty minutes afterward have him empty his bladder and discard the urine. Then inject intramuscularly 1 c.c. of sterile phenolsulphonephthalein solution. This may be secured in ampules from Hynson, Wescott and Dunning.

3. In exactly one hour and ten minutes from the time of the injection have the patient empty his bladder and save all the urine. The ten minute

*University of Minnesota, University Farm, St. Paul, Minnesota.
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period represents the usual time which elapses between the injection and the first appearance of the dye in the urine.

4. In two hours and ten minutes after the injection, the bladder is again emptied and the urine saved in a separate container.

5. To estimate the output of phenolsulphonephthalein, the volume of each of the one hour portions of urine is recorded; to each of the two portions is added sufficient 10 per cent sodium hydroxide solution to bring out the maximum purplish red color, and each portion is made up to a volume of 1000 c.c.

A standard is prepared by adding 1 c.c. of the phenolsulphonephthalein solution to about 800 c.c. of water; this is alkalinized by sodium hydroxide and diluted to 1000 c.c. The unknown is read against the known in a colorimeter. Usually, percentage solutions of the known are made up and compared against the unknown.

An adaptation of this method was applied to cattle but it was found that when the urine was treated with sodium hydroxide, and diluted to 1000 c.c. in each case it was so cloudy that no colorimetric reading could be made. This cloudiness could not be removed by any filtering process that would not take out the dye.

It was the belief that this cloudiness was due to the suspended sulphates and phosphates in the urine, which are contained in abundance in ox urine.

After trying several methods of precipitating these sulphates and phosphates without success, it was suggested that barium hydroxide be tried, as these constituents are precipitated by barium hydroxide. Accordingly one part by volume of a saturated solution (5 per cent) of barium hydroxide, was added to five parts of urine, after which the material was filtered with the result that the filtrate maintained the original color and was a clear transparent liquid.

A standard solution was then made up using 100 c.c. of a saturated barium hydroxide solution, dissolving one c.c. of the phenolsulphonephthalein solution and the volume made up to 1000 c.c.

It is suggested that this method might have an application in the routine procedure of the phenolsulphonephthalein renal function test in its application to humans.

A NEW METHOD FOR STAINING ELASTIC FIBERS*

By Joseph M. Thüringer, M.D., Norman, Okla.

AMONG the various stains for the demonstration of elastic tissue the Resorcine-Fuchsin method of Weigert[1] and the Orcein method of Unna-Tänzer with their numerous modifications are familiar to every worker. The preparation of the Resorcine-Fuchsin stain is a somewhat tedious process requiring much time though the staining rarely takes more than an hour.

The Orcein method of Tänzer[2] or Unna[3] requires staining from six to twenty-four hours at room temperature although it may be hastened by staining in a moderately warm incubator or over a flame until the stain thickens.

Harris[4] makes use of "Elasthaematin" which is a stain composed of hematoxylin and aluminum chloride, ripened with mercuric oxide and acidulated with HCl.

The above mentioned and numerous other methods while yielding good results are open to many objections the principal ones of which are:

1. That a special stain must be kept on hand.
2. The loss of time in the preparation of the stain.
3. The length of time required for staining.
4. Some of the methods are not suitable for the simultaneous preparation of a large number of sections or slides.
5. Since the stain is not frequently employed it is usually unfit for use when desired.

To overcome these objections I have looked for a method which would permit that the ordinary routine hematoxylin-eosin stain be adopted for this special purpose.

The sections are given a preliminary treatment as follows:

(a) Place in one-half of 1 per cent phosphotungstic acid for five minutes.
(b) Rinse well in distilled water.
(c) Stain deeply in Mann's hematin three to five minutes.
(d) Differentiate in 2½ per cent acetic acid in water.
(e) Rinse in distilled water.
(f) Stain with eosin.
(g) Dehydrate and clear.

The entire procedure varies from a routine hematoxylin-eosin stain only in the preliminary treatment of the sections in one-half of 1 per cent phosphotungstic acid solution in water.

Mann's hematin is the formula employed in my laboratory for routine work. It is essentially similar to Ehrlich's acid hematoxylin but is made up

*Department of Histology, University of Oklahoma, Norman, Oklahoma.
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from hematin instead of hematoxylin. It is ready for instant use and five-year-old solutions on hand show no signs of deterioration.

Formula for Mann's acid hematin

Hematin	1.25 gm.
Glacial acetic acid	10.00 c.c.
Glycerine	100.00 c.c.
Absolute alcohol	100.00 c.c.
Potassium-alum sulphate	10.00 gm.
Water	100.00 c.c.

Dissolve the powdered hematin in the acetic acid; if necessary add 50 c.c. of absolute alcohol. Then add the glycerine, rinsing the measuring cylinder with the remaining 50 c.c. of the absolute alcohol, which also add.

Dissolve the alum in the water with the aid of heat and when partly cooled mix with the above and filter. The stain is now ready for use.

The elastic fibers are stained deep blue and appear in great contrast to the general pink of the remainder of the section. The nuclei stain red but are unobtrusive. The chromatin granules vary from light brown to blue.

The elastic lamina of arteries show beautifully as well as the most delicate elastic fibers scattered throughout the tissue.

Delafield's hematoxylin may be used if it is desired to show nuclei with greater contrast.

Hansen's hematoxylin is especially recommended if a brilliant nuclear stain is desired at the same time. The contrast between the elastic fibers and other tissue elements, however, is greatly diminished.

Phosphomolybdic acid must not be used in place of phosphotungstic acid since its "affinities" lie more in the direction of the collagen fibers which stain very deeply with the hematoxylin after preliminary treatment with dilute solutions.

SUMMARY

1. The above method gives a very specific stain for elastic tissue.

2. Any section which takes a good hematoxylin-eosin stain will yield good results.

3. Any of the common fixatives may be used, Zenker's, Helley's, formalin, alcohol, etc.

4. No special stain has to be prepared. It is only necessary to "mordant" sections for five minutes in one-half of 1 per cent phosphotungstic acid in water before staining with hematoxylin.

5. This method gives uniformly good results with paraffin, celloidin or frozen sections.

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EDITORIALS

Heart Block

THAT there is romance in the history of medicine no one may deny although the story may not have the content or even the form of the everyday one of love and adventure. But the romances of medicine are not as popular, are not as well known, are not, perhaps, as easily understood as the "best sellers" of current fiction. If this were not so the works of Brown-Séquard would be as well known as the Henty books or the Nick Carter series. Perhaps Gertrude Atherton will popularize certain aspects of medical research from the beginning with *Black Oxen*. It is a pity that Franz' *Through the Ivory Gate* is not read more. It is to be regretted that Freud is looked upon as erotic rather than as romantic.

The romances of medicine appeal perhaps only to the active workers in science. Sometimes when they are read by the student they are the immediate cause of a new career in science. Often they speed up the enthusiasm of the middle-aged routine worker or bring new thrills to the tired researcher.

Only occasionally someone writes an essay or even sometimes a book that is positively thrilling. Such essays are certain ones of Osler, of Daniel Drake, of Darwin, of Huxley. Such books are exemplified in Vallery-Radot's *Life of Pasteur* and in Cushing's *Life of Osler*. The whole career of Paracelsus is romantic even when one subtracts the halo Browning built about it. It was romantic to Edith Wharton whose poem on Paracelsus is a jewel. The more popular medical stories belong to the field of the practice of medicine. Few of them relate to the laboratory aspects of scientific labor. It seems popularly true that the sentimental relations of physician and patient should be the central theme as exemplified in *Rab and His Friends* and very many other stories. Perhaps *Black Oxen* is evidence of change in a new direction, and *Dracula* in another. At any rate even in addresses for physicians themselves it is usual to call attention to the results that they have had with the products of laboratory research, which is to say, the end-result is apostrophized rather than the trials of production. Hence it is perhaps that Lister is better known even to physicians than Pasteur or Liebig, and that few physicians know Theobald Smith, the pioneer in the study of transmission of disease by insects. When both aspects, the experimental (the so-called theoretical and underpaid) and the therapeutic (the so-called practical and overpaid) are used together, a complete scientific romance may be the result as in the gorgeously true *Arrowsmith*, a story of the "main street" that runs about the Olympus of medicine. The ordinary accounts of the trials, tribulations and successes of medicine are like the recent address of Haggard oratorical patchworks elaborated from bits of biography used for the purpose of professional sentimental exaltation. They belong with the Fourth of July orations.

In days gone by scientific progress was largely an individual affair, each step in advance the result of the efforts of a single worker testing his vision of what might be. Now it is become a matter of teamwork, of cooperation in research. Modern science depends for progress upon this teamwork which is the new factor added to the old one of intellectual liberty. Sabin has made a stimulating plea for this combination.

One of the most recent romantic fragments and one which shows the value of cooperative research, is to be found in a yearbook! In his report for the Department of Embryology of the Carnegie Institution of Washington, Dr. Streeter reports certain researches on the physiology of the heart. Let me quote: "It was found by Mr. Johnstone that delicate ligatures could be placed at different levels around the beating hearts of chick embryos and that he could thus functionally isolate the different parts of the heart tube, producing what is known in the adult as *heart block*, a condition where the subdivisions of the heart beat independently of each other, the coordination normally existing between ventricle and atrium being lost. Since this could be done in embryos of 2, 3, and 4 days' incubation, it became possible for Mr. Johnstone to study contraction of embryonic heart-muscle before the problem is complicated by the ingrowth of nerve fibers, and where the only conducting system is the muscle itself. Further than that, by altering the level of the ligatures, the different parts of the heart tube could be

observed, both separate and connected, and thus the effect of one part on another could be determined for different stages of growth.

"Mr. Johnstone found that when a ligature is tied at the atrio-ventricular junction, the atrium continues to pulsate, while the ventricle and bulbus, after stopping for a moment, begin again to contract, but at a slower rate and more irregularly than that portion of the heart on the other side of the ligature—that is, a definite heart block is produced. If, after the ventriculo-bulbar segment has resumed its contracting, another ligature is placed around the heart at the junction of the ventricle and the bulbus arteriosus, the latter stops contracting while the ventricle continues without apparent change. The bulbus in these experiments sometimes fails to beat again, but if pulsation is reestablished it is after a longer interval and at a slower rate than in the isolated ventricle.

"At present there is some uncertainty as to the exact location of the junction of the sinus venosus with the atrium in these early stages, and this is one of the problems that now faces us. Mr. Johnstone placed a ligature in this general region and all that part of the heart tube cranial to the ligature (atrium, ventricle and bulbus), after a brief cessation, resumed pulsations, maintaining the regular sequence of atrium, ventricle, and bulbus, though at a slower rate than before tying the ligature. It would thus appear that the ultimate and regulative control of the heart beat must be in the sinus region.

"Further experiments were done in which the ligatures, after being firmly tied, were removed, leaving a crushed line of constriction in the cardiac tube that physiologically isolated the portions on either side of the constriction, producing heart block in accordance with the level at which the crushing occurred.

"Dr. Lewis has studied the initiation and character of the early rhythmic contractions of the heart by removing this organ from 2- and 3-day chick embryos and observing its behavior in Locke's solution, where it maintains fairly regular automatic pulsations for several hours at room temperature (26° C.), varying in different hearts from 40 to 80 per minute, which is about one-third of that in the freshly opened egg. If the temperature is gradually increased the rate increases, going from 26° to 30°, about 4 beats per minute, from 30° to 35° from 4 to 8 beats per minute, and from 35° to 40° from 4 to 20 beats per minute. The rate of the isolated heart varies definitely according to certain environmental conditions, such as temperature, carbon-dioxide tension, hydrogen-ion concentration, and salt content of medium, and also upon mechanical stimulation.

"In studying the effect of changes of temperature on contraction phenomena of these isolated hearts, Dr. Lewis found that the heart block which Mr. Johnstone had effected by ligatures could be brought about simply by an abrupt rise in temperature. For example, when a heart beating regularly at 92 per minute at 27° C. was placed in a warm box at 38° C., after three minutes the atrium was beating at 120 and the ventriculo-bulbar part at 12 per minute; and when returned to room temperature, within ten minutes the whole heart was found beating synchronously again at 92 per minute. There

was some variation in the behavior of the individual specimens as to the degrees of temperature necessary and the amount and character of the disharmony, but in general the effect was as striking as the illustration given. With few exceptions the atrial end was most resistant and beat most rapidly and the ventricular and bulbar portions, in that order, exhibited a much slower rhythm and showed more tendency to be intermittent. The heart block of the clinicians has been supposed to be due to injury to the atrio-ventricular conduction bundle. Since these young embryonic hearts contain no such bundle, it cannot be the explanation here. Nor is it due to any serious injury of the atrio-ventricular or ventriculo-bulbar junctions, since the condition disappears when the hearts are brought back to room temperature. The explanation must rest in the physiologic differences existing between the muscle cells of the atrium, the ventricle and the bulbus. Apparently these physiologic differences are increased through the circumstances of the experiment and the different parts of the heart are rendered more sensitive to thermal and other changes. It is certainly not temperature alone that causes heart block, because the same temperature in the egg, with the heart in the normal environment, yields a synchronously beating heart.

"The inherent difference in the constituent cells of the atrial, ventricular and bulbar portions of the cardiac muscle-sheet is best shown by dividing the isolated heart into these three parts and studying them in Locke's solution. Dr. Lewis made records of such preparations, lying side by side, under various conditions of temperature and in open and closed chambers. This latter point makes a difference, for when the observing chamber is closed with a cover-glass the rate is accelerated, due presumably to increased carbon dioxide tension. It is hoped that this important point can be definitely determined. When kept at room temperature it was found, in confirmation of Mr. Johnstone's ligature experiments, that the atrium always beats faster than the ventricle and the ventricle faster than the bulbus. The rate of the atrium approaches closely that of the entire heart. The rate of the ventricle, on the other hand, is usually less than half that of the atrium for the 2-day hearts, and considerably less than that for the 3-day hearts. The ventricular rhythms are apt to be more irregular than the atrial. The pulsations of the bulbus often fail entirely and are usually few in number and irregular when they do occur. All of these things make it quite evident that the relatively fast rate of the ventricle and bulbus in the intact heart is dependent upon stimulus from the atrium. When these parts are separated they show less spontaneity in older stages than younger ones. In other words, the muscle cells of the ventricle and bulbus tend to lose their spontaneous rhythmicity with age."

Isn't that romance?

—*P. G. W.*

Cancer and the Filterable Virus

WITH the arrival of the transactions of the ninety-third annual meeting of the British Medical Association we find instructive details on the work of Gye and Barnard which has recently been heralded in the lay press. Dr. William Ewart Gye of the Department of Experimental Pathology of the National Institute for Medical Research at Hampstead, presented the results of his studies before the section on pathology and bacteriology.

Years ago, contagious disease was thought to depend for its origin merely on the juxtaposition of host and parasite. While this still holds for many infections, exceptions do exist. Tetanus bacilli washed free from toxin are harmless. But if sufficient toxin is introduced together with the germ, tetanus ensues. The same is true of B. welchii infections. Yet a third factor sometimes plays a part, the factor of the body tissues or tissue resistance. Fifty per cent of a population may be carriers of the meningococcus but remain free from meningitis. The same occurs in amebiasis, diphtheria, typhoid fever and other diseases.

Cancer has been held to be a specific disease entity in part because a tumor arising in one animal can only be transmitted to another animal of the same species and only after the injection of living cancer cells. On transplantation the characteristics of the parent tumor are faithfully reproduced. Opposition to the infection hypothesis has been made chiefly by those students of cancer research who have been interested rather in morphologic studies and whose chief concern has been the specificity of the different types of malignant disease. All this has been considered evidence against the germ theory of cancer, but Gye points out one important exception among animal and experimental cancers. This is the chicken sarcoma discovered by Peyton Rous in 1911 which was transmitted by the implantation of dead sarcoma cells or even by the injection of cell-free filtrates of the sarcoma. Two different chicken tumors have since been discovered by Rous, in each of which the sarcoma produced by filtrates always retained the same characteristics as the original. Two are spindle-cell sarcomas and the third an osteochondrosarcoma.

Gye has discussed at considerable length the difficulties and drawbacks associated with the study of so-called filterable viruses. Much has been done with these forms of life since Loeffler and Frosch discovered, in 1898, the first filterable virus in foot and mouth disease, but many of our concepts still appear erroneous. By many filterability is still considered the final criterion. There are, however, other factors which are even more important. Thus in herpetic encephalitis the saline filtrate from the herpetic vessicle is active, while the brain tissue thoroughly ground with sand and diluted with saline is noninfective after filtration. He suggests that here the virus has become so firmly attached to the brain tissue that it cannot be separated by ordinary mechanical processes. McCartney disagrees on this point, ascribing the noninfectivity of brain tissue filtrate rather to the presence of lipoid material in the macerated solution which clogs the pores of the filter. He points out that after preliminary incubation in glucose

broth, the virus passes from the tissue into the surrounding medium and will then pass through the pores of a Berkefeld filter.

Gye points out that in vaccinia, which is undoubtedly a virus infection, the filtrate from calf lymph does not contain the virus. He suggests that perhaps the virus becomes attached to the contaminating cocci or that it has the property of being adsorbed onto the surface of the filter. Gordon, who has done much work on the virus of vaccinia, in discussing this phase of Gye's communication, states that if *sufficient* filtrate be used the presence of virus in small quantities can be proved. If the suspension of virus be autolyzed before filtration, the virus will come through. It may also be liberated from the cells by preliminary trypsinization. He also records that the virus is readily adsorbed by the filter itself and concludes that there can be no reasonable doubt that vaccinia is due to a filterable virus. Coplans points out the marked absorptive properties of recently baked filters.

But, returning to the work of Gye, he has had no great difficulty in filtering what appears to be the virus of chicken sarcoma. This is confirmatory of Rous' work. Success in filtration requires a sufficiently great dilution and the use of salt solution rather than distilled water. Hydrogen-ion concentration appears also to be a factor governing filterability. Acidity hinders filtration while alkalinity makes the process easier. Apparently the properties of the liquid in which the virus is suspended play an important part.

The specificity of tumor cells is remarkable. But just as remarkable is the fact that cell-free filtrates of chicken sarcoma are equally specific. They produce tumor in no other bird than the hen and best in that particular variety from which they were first isolated, the Plymouth Rock.

The author suggests that in cancer as in tetanus there may be two essential factors, the filterable virus and the tumor tissue itself or at least some derivative of the tumor cells. He brings forth evidence that a chemical accessory factor is necessary for the production of tumors. The Rous sarcoma tissue becomes noninfective in primary broth culture within from two to seven days. Gye felt that this might be due not to the death of the virus but to the disappearance of an accessory chemical factor. Rous had observed that chloroform and certain antiseptics destroy the infectivity of filtrates, presumably by killing the virus. Gye, therefore, treated a fresh filtrate with chloroform to destroy the virus. Theoretically, the *chemical factor* was still present in the filtrate. This chloroform treated filtrate when injected into a hen caused no tumor. A noninfective primary culture presumably containing *the virus* also caused no growth. The two injected together in equal parts did result in tumor growth.

After centrifuging a filtrate at 9,000 revolutions per minute for two hours, the sediment was found to be infective while the upper layers were not. This suggests strongly the particulate character of the virus. If now the sediment is washed with saline and repeated centrifuging the sediment which still contains the virus but none of the chemical factors in the solution, is no longer infective. Neither is the supernatant fluid from the original centrifugalization which presumably contains chemical factor but no virus. Both together, however cause tumor growth. One of the factors is thrown

down in the centrifuge while the other is not. This suggests that the latter is indeed a chemical substance. Neither operating alone will cause sarcoma.

Gye believes that since the virus alone is not infective, the specificity of the Rous tumor must depend upon a chemical factor derived from the tumor cells. That it is actually derived from the tumor cells is suggested by the observation that subcultures are not infective. It is only the original or primary culture which contains tumor tissue that continues infective when used alone. Gye believes that the virus in the three forms of Rous chicken sarcoma is probably the same in each case, while the chemical factors are different. The factor is a cell derivative which may be separated from the cells.

He appears to have demonstrated satisfactorily that the particulate factor, the virus, will multiply in vitro. With the aid of KCl broth and chicken embryo subcultures have been made from the primary culture. A fifth subculture will have diluted the original inoculum one thousand billion times. While an eighth subculture causes no tumor, and a chloroform-treated filtrate of a primary culture (containing the chemical factor alone) causes no tumor, this treated filtrate *plus* the eighth subculture caused the usual tumor in the customary length of time.

The work on mammalian tumors has been unusually interesting. A spindle-cell mouse sarcoma, a rat sarcoma, a mouse carcinoma and a rat carcinoma were studied.

While the filtrate from mouse sarcoma will not cause sarcoma in the mouse, a recent unfiltered primary culture thereof will produce a growth. However, if the primary culture be maintained under *strict* anaerobic conditions, the filtrate *will* cause tumor growth. Thus mammalian sarcomas can likewise be transmitted with a cell-free filtrate and they thereby resemble the Rous chicken sarcoma. The difference lies in that the accessory chemical factor is either more abundant or more stabile in the chicken sarcoma. The evidence points to a destruction of the chemical factor by oxidation and it may be that strict anaerobic conditions preserve the chemical factor in greater quantities.

Gye has thus shown that mammalian sarcomas behave like chicken sarcomas provided the chemical factor is present in sufficient quantity and is not destroyed by manipulation.

In his work on the rat sarcoma and the rat and mouse carcinomata, the author employed the chemical factor obtained from the Rous chicken sarcoma. He did this primarily because the chemical factor appears to be more abundant in the chicken sarcoma. The cultures used were from the mammalian tumors and theoretically contained the virus and little or no specific chemical factor. The injection into chickens of *mammalian* virus with the chicken sarcoma chemical factor causes a tumor growth that can in no way be distinguished from the original Rous chicken sarcoma. Gye concludes that the virus obtained from each of these five sources reacted alike and that the chemical factor determined the specificity of the growth. He does not give protocols describing the injection of mammalian virus and Rous chemical factor into mammals. This should serve as a control for the injections into birds.

Three human tumors have been studied. The culture of the first from a scirrhous carcinoma of the breast was contaminated. The second, from a sarcoma of the thigh gave negative results. Cultures were made from the third, an adenocarcinoma of the breast, and after injection into a chick of this culture, together with the Rous chemical factor, a tumor resulted which was indistinguishable from the Rous tumor. As a control the chemical factor alone did not cause tumor.

It should be noted that cultures rather than filtrates of the mammalian tumors were used in the inoculation experiments.

Gye contrasts his specific factor with the commonly known aggressions. The toxin of B. welchii may be taken as example of the latter. Bacilli washed free from this toxin are harmless to mice. Washed cultures of B. welchii to which has been added a sublethal dose of the toxin, produce a characteristic spreading gangrene with toxemia. The disease is characteristic of B. welchii infection. This same toxin added to vibrion septique causes the disease characteristic of vibrion septique infection. Toxin-free harmless tetanus spores mixed with sublethal doses of B. welchii toxin caused tetanus. The specificity of these diseases depends upon the germ while the chemical agent is nonspecific. This would appear the reverse of the situation with Gye's cancer virus.

He accepts in part the suggestion made by various workers that the filterable viruses act upon cells in a different manner from the larger microbes. They are probably intracellular during their existence in the host in contrast to the bacterial microorganisms which exist chiefly in the connective tissues.

He feels that the virus of chicken sarcoma is corpuscular in nature, particularly since after prolonged centrifugalization the lowermost layers of the fluid cause more rapid and extensive growth than the uppermost. (The rapidity of appearance and growth of this tumor depends on the amount of virus implanted.)

He finds that the organism cannot be stained and examined with the ordinary microscope or with the routine staining methods such as Giemsa's or polychrome methylene-blue. The microscope is of value only in revealing the presence of contaminating germs. Barnard, however, has constructed an ultramicroscope employing ultraviolet rays, with which he has clearly photographed objects less than 0.2 micron diameter. He describes the virus as a small spheroid, which appears to reproduce by a process somewhat akin to "budding." Small particles develop at the periphery of the spheroid and gradually become loosened after remaining for a time attached by a very fine filament. The particles appear later to become new spheroids. Photographs show a magnification of 2200. The author estimates the diameter of the particles as less than 0.075 micron. The spheroids have roughly five times this diameter.

In view of recent statements intimating that Barnard was a merchant who had happened upon his discovery somewhat by accident, it should be noted that he has made various contributions in the medical literature, that

he is associated with the National Institute for Medical Research and is a Fellow of the Royal Society.

Gye believes that his virus is not a phase in the life history of a larger organism although his observations so far naturally cannot be conclusive on this point.

Several pertinent points were raised by Dr. J. A. Arkwright in his discussion of Dr. Gye's paper. Dr. Arkwright said: "As a general comment on the discussion it seems to me that there should be no assumption that the viruses known as "filter-passers" are all of a similar nature. In regard to many of them it may be said that we have little reason to regard them as similar except our ignorance of their nature, including our inability to see them. Next, I should like to express my unbounded admiration for the work of Dr. Gye and Mr. Barnard on the cause of cancer. This does not mean that I think there will be no criticism of their interpretation of the experiments they have published. Whilst their facts are no doubt correct the hypothesis will, I feel sure, be subjected to critical examination. There are two points on which I should like to ask Dr. Gye to give us further enlightenment. The first question, which it seems almost certain he will answer in the affirmative, is whether the experiments, in which Rous' fowl sarcoma was produced by the inoculation of a chloroform-treated 'sand filtrate' of one of these tumours together with a 'subculture' of the Rous 'virus,' were controlled by giving parallel injections of material in which uninoculated culture medium containing fresh chicken embryo was substituted for the 'subculture.' Another point which strikes one is the resemblance between the chloroformed-sarcoma extract, which Dr. Gye does not regard as living and calls the 'specific chemical factor,' and some of the so-called 'filter-passing viruses' which are associated with various other diseases. Some of these are remarkably resistant to adverse conditions. For example, the virus of vaccinia is well known to have a high resistance to chloroform and ether, and the virus of foot-and-mouth disease is known to resist drying over sulphuric acid, and was found by Loeffler sometimes to withstand 1 per cent carbolic for many weeks. If the 'specific factor' is really a simple chemical substance, as Dr. Gye believes, it is remarkable that it should be able to determine the kind of cell in the new growth resulting from an inoculation and the kind of animal in which the virus can cause growth."

Dr. Gye said: "The answer to Dr. Arkwright's first question is 'Yes.' Parallel experiments have been done many times with negative results. The second question is more difficult. The interpretation put upon the experiments has been founded upon: first, cultures from chloroformed extract fail; secondly, direct dilution experiments are all favourable to the interpretation given; and thirdly, the assumption that the effective factor is another virus again leads us to the position that strictly specificity is dependent upon a virus. In short, the simplest and most direct interpretation has been put forward, and this is supported by available facts."

The work of Gye and Barnard is stimulating. Their experiments must of course be confirmed. If such confirmation is forthcoming it matters little whether the explanatory hypothesis will require modification. The essential

point is that, while the morphologic pathologists have to a certain extent reached an impasse and the outlook for early progress has appeared doubtful, the resurrection of the infection hypothesis opens new avenues of approach and study.

Work on filterable viruses is a most delicate procedure and being dependent on a large number of factors, requires a greater variety of checks and counter checks than the authors have as yet applied.

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—*W. T. V.*

Rural Hospitals

With the purpose of improving rural medical nursing and hospital care, the Commonwealth Fund of New York is offering to assist in the building of one hospital in a rural section. Certain conditions are laid down governing the distance from existing hospitals, number of physicians for staffing the hospital, character of highways and transportation lines, willingness and ability of the community to defray deficits from operation. The Fund offers to pay two-thirds of the cost of construction and equipment.

If the experience in building one such hospital seems to warrant an extension of the program, the Fund will consider making a like offer to other districts needing a hospital. Applications are now being received, and considered. It is probable that a decision as to the first unit will be made in the early fall.

Erratum

On page 496, March, 1925, issue, Dr. Wadsworth's article, the third line in the tabular matter at the bottom of the page should read:—1 c.c. 4N HCl = 40.00 c.c. N/10 HCl

The American Society of Clinical Pathologists

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Dallas Chosen for Next Convention

Responding to the cordial invitation extended by our colleagues in Texas, the Executive Committee of the American Society of Clinical Pathologists and the newly elected officers have unanimously chosen Dallas as our meeting place for the 1926 convention. The date has not as yet been definitely settled but it will permit our members to attend the annual American Medical Association meeting which will be held in the same city.

The selection of Dallas for our next convention is a well merited tribute to the Texas contingent of the American Society of Clinical Pathologists who were among the most active in the organization of our society and the first to form a state association of pathologists, thus stimulating the formation of our national organization.

Socialization of Clinical Pathology

One of the topics under consideration at the business session of the last Convention of American Society of Clinical Pathologists which elicited considerable and spirited discussion was the encroachment of state and municipal laboratories in the domain of the clinical pathologist. The socialization of medicine has been going on apace. One hears protests from the profession on the invasion of State Medicine in the field of general practice. The complaint is not limited to the medical profession. In industrial, manufacturing, and commercial spheres we hear a good deal of state interference with private initiative.

It is not within our province to enter here into political economy and dilate on the relative merits of state versus individual operation. The business people can take care of themselves. The medical profession at large is

making itself felt through the American Medical Association against the competition of state medicine.

Clinical pathology will have to fight for its rights in the competitive struggle. The principle here involved is not necessarily the vulgar bread and butter question but the interest of the patient. Socialization of clinical pathology with the concomitant low compensation will not attract the best talent, who will seek other fields. The result will be an incompetent personnel with consequent injury to the welfare of the patient.

Here is where organization can be effective in combating socialization of clinical pathology. The appointment of a Committee by the American Society of Clinical Pathologists to study the problem is a step in the right direction.

Society Notes

The Society maintains a Service Bureau for its members. Any clinical pathologist wishing to make a change should communicate with the Secretary.

Kindly report any change of address to the Secretary.

The next annual Convention of the American Society of Clinical Pathologists will be held in Dallas, Texas, April 15, 16, 17, 1926.